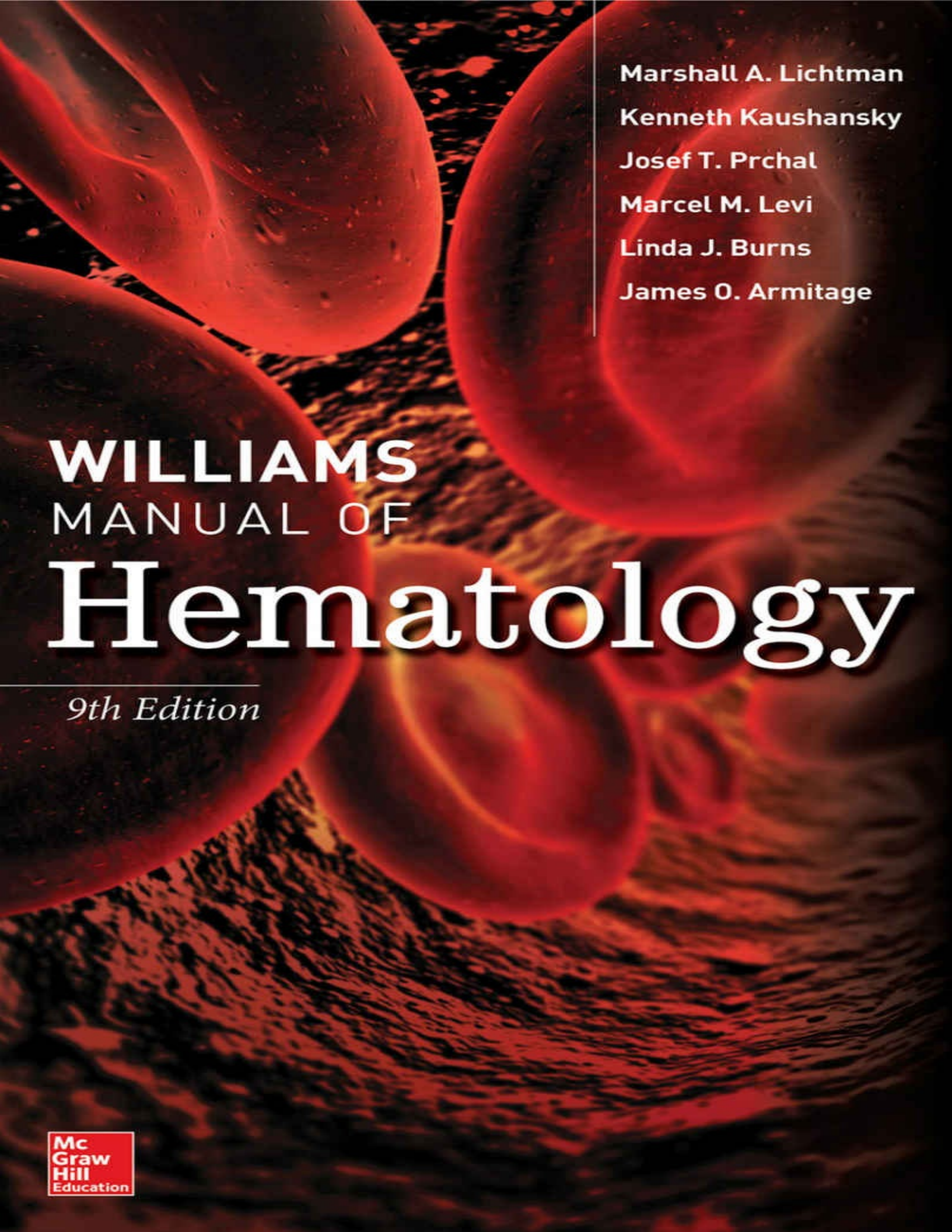
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Williams
Manual of Hematology

Ninth Edition

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Williams Manual of Hematology, Ninth Edition

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CONTENTS

PREFACE

Williams Manual of Hematology provides a convenient and easily navigable précis of the epidemiology, etiology, pathogenesis, diagnostic criteria, differential diagnosis, and therapy of blood cell and coagulation protein disorders. The 93 chapters in the *Manual* are a distillation of the disease- and therapy-focused chapters of the ninth edition of *Williams Hematology*. The *Manual* is a handbook, but it is comprehensive. It is organized into 12 parts, paralleling the ninth edition of *Williams Hematology,* yet of a size that permits it to serve as a companion to the physician in the hospital or clinic. It can be used as a hard copy carried in one's coat pocket or, more deftly, as an app on one's smart phone or tablet.

We have included chapters on the classification of red cell, neutrophil, monocyte, lymphocyte, and platelet disorders and of diseases of coagulation proteins to provide a framework for considering the differential diagnosis of syndromes that are not readily apparent. Also included are numerous tables that contain diagnostic and therapeutic information relevant to the diseases discussed. Detailed chapters describing the features of individual myeloid and lymphoid malignancies provide a guide to diagnosis, staging, and management. Chapters on the manifestations, diagnostic criteria, and therapy of hereditary and acquired thrombophilia consider the role hematologists play in diagnosing and managing this important mechanism of disease. Descriptions of diseases of red cells, neutrophils, monocytes, macrophages, lymphocytes, platelets, and coagulation proteins and their management leave no gaps and meet the needs of the busy hematologist, internist, or pediatrician. In addition, this handbook is very useful for advanced practice professionals, medical and pediatric residents and subspecialty fellows, and medical or nursing students because of its succinct clinical focus on diagnosis and management.

For many tables reproduced in the *Manual*, the reader can find explicit citations documenting those entries in the concordant chapter in the ninth edition of *Williams Hematology.* In addition, where helpful, images of blood or marrow cell abnormalities or external manifestations of disease are included. Each chapter ends with an acknowledgment of the authors of the relevant chapter in the ninth edition of *Williams Hematology,* including the chapter title and number for easy cross-reference to that comprehensive text.

The publisher prints a caution in the *Manual* that admonishes readers to verify drug doses, routes of administration, timing of doses, and duration of administration and to check the contraindications and adverse effects of drugs used to treat the diseases described. We reemphasize that these often complex diseases require direct participation and close supervision of an experienced diagnostician and therapist. This oversight should be provided by a person who is able to individualize therapy depending on the nature of the expression of the primary hematological disease, the patient's physiological age, and the presence of coincidental medical

conditions, among other factors.

The authors acknowledge the valuable assistance of Marie Brito at Stony Brook University, Kim Arnold at the University of Nebraska, and, notably, Susan Daley at the University of Rochester, who entered tables and figures into the chapters, managed the administrative requirements in the preparation of the *Manual*, and coordinated communication among the six of us and McGraw-Hill. We also acknowledge the encouragement and support of Karen Edmonson, Senior Content Acquisitions Editor, and Harriet Lebowitz, Senior Project Development Editor, at the Medical Publishing Division, McGraw-Hill Education.

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PART I

INITIAL CLINICAL EVALUATION

CHAPTER 1
Approach to the Patient

FINDINGS THAT MAY LEAD TO A HEMATOLOGY CONSULTATION

Table 1–1 lists abnormalities that often require an evaluation by a hematologist.

TABLE 1–1 FINDINGS THAT MAY LEAD TO A HEMATOLOGY CONSULTATION

Decreased hemoglobin concentration (anemia)
Increased hemoglobin concentration (polycythemia)
Elevated serum ferritin level
Leukopenia or neutropenia
Immature granulocytes or nucleated red cells in the blood
Pancytopenia
Granulocytosis: neutrophilia, eosinophilia, basophilia, or mastocytosis
Monocytosis
Lymphocytosis
Lymphadenopathy
Splenomegaly
Hypergammaglobulinemia: monoclonal or polyclonal
Purpura
Thrombocytopenia
Thrombocytosis
Exaggerated bleeding: spontaneous or trauma related
Prolonged partial thromboplastin or prothrombin coagulation times
Venous thromboembolism
Thrombophilia
Obstetrical adverse events (eg, recurrent fetal loss, stillbirth, and HELLP* syndrome)

*__H__emolytic anemia, __e__levated __l__iver enzymes, and __l__ow __p__latelet count.
Source: *Williams Hematology*, 9th ed, Chap. 1, Table 1–1.

The care of a patient with a hematologic disorder begins with eliciting a medical history and performing a thorough physical examination. Certain parts of the history and physical examination that are of particular interest to the hematologist are presented here.

HISTORY OF THE PRESENT ILLNESS

- Estimation of the "performance status" helps establish the degree of disability and permits assessment of the effects of therapy (Tables 1–2 and 1–3).
- Drugs and chemicals may induce or aggravate hematologic diseases; drug use or chemical exposure, intentional or inadvertent, should be evaluated. One should inquire about professionally prescribed and self-prescribed drugs, such as herbal remedies. Occupational exposures should be defined.
- Fever may result from hematologic disease or, more often, from an associated infection. Night

sweats suggest the presence of fever. They are especially prevalent in the lymphomas.

- Weight loss may occur in some hematologic diseases.
- Fatigue, malaise, lassitude, and weakness are common but nonspecific symptoms and may be the result of anemia, fever, or muscle wasting associated with hematologic malignancy or neurologic complications of hematologic disease.
- Symptoms or signs related to specific organ systems or regions of the body may arise because of involvement in the basic disease process, such as spinal cord compression from a plasmacytoma, ureteral or intestinal obstruction from abdominal lymphoma, or stupor from exaggerated hyperleukocytosis in chronic myelogenous leukemia.

TABLE 1–2 CRITERIA OF PERFORMANCE STATUS (KARNOFSKY SCALE)

Able to carry on normal activity; no special care is needed.	
100%	Normal; no complaints, no evidence of disease
90%	Able to carry on normal activity; minor signs or symptoms of disease
80%	Normal activity with effort; some signs or symptoms of disease
Unable to work; able to live at home, care for most personal needs; a varying amount of assistance is needed.	
70%	Cares for self; unable to carry on normal activity or to do active work
60%	Requires occasional assistance but is able to care for most personal needs
50%	Requires considerable assistance and frequent medical care
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.	
40%	Disabled; requires special care and assistance
30%	Severely disabled; hospitalization is indicated though death not imminent
20%	Very sick; hospitalization necessary; active supportive treatment necessary
10%	Moribund; fatal processes progressing rapidly
0%	Dead

Source: *Williams Hematology*, 9th ed, Chap. 1, Table 1–2.

TABLE 1–3 EASTERN COOPERATIVE ONCOLOGY GROUP PERFORMANCE STATUS

Grade	Activity
0	Fully active; able to carry on all predisease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature (eg, light housework, office work)
2	Ambulatory and capable of all self-care but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited self-care; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any self-care; totally confined to bed or chair
5	Dead

Source: *Williams Hematology*, 9th ed, Chap. 1, Table 1–3.

FAMILY HISTORY

- Hematologic disorders may be inherited as autosomal dominant, autosomal recessive, or X chromosome–linked traits (see *Williams Hematology*, 9th ed, Chap. 10). The family history is crucial to provide initial clues to inherited disorders and should include information relevant to the disease in question in grandparents, parents, siblings, children, maternal uncles and aunts, and nephews. Careful and repeated questioning is often necessary because some important details, such as the death of a sibling in infancy, may be forgotten years later.
- Consanguinity should be considered in a patient who belongs to a population group prone to marrying family members.
- Absence of a family history in a dominantly inherited disease may indicate a de novo mutation or nonpaternity.
- Deviations from Mendelian inheritance may result from uniparental disomy (patient receives two copies of a chromosome, or part of a chromosome, containing a mutation from one parent and no copies from the other parent) or genetic imprinting (same abnormal gene inherited from mother has a different phenotype than that inherited from father as a result of silencing or imprinting of one parent's portion of DNA) (see *Williams Hematology*, 9th ed, Chap. 12).

SEXUAL HISTORY

- One should obtain the history of the sexual preferences and practices of the patient.

PHYSICAL EXAMINATION

Special attention should be paid to the following aspects of the physical examination:

- *Skin:* cyanosis, ecchymoses, excoriation, flushing, jaundice, leg ulcers, nail changes, pallor, petechiae, telangiectases, rashes (eg, lupus erythematosus, leukemia cutis, cutaneous T-cell lymphoma)
- *Eyes:* jaundice, pallor, plethora, retinal hemorrhages, exudates, or engorgement and segmentation of retinal veins
- *Mouth:* bleeding, jaundice, mucosal ulceration, pallor, smooth tongue
- *Lymph nodes:* slight enlargement may occur in the inguinal region in healthy adults and in the cervical region in children. Enlargement elsewhere, or moderate to marked enlargement in these regions, should be considered abnormal
- *Chest:* sternal and/or rib tenderness
- *Liver:* enlargement
- *Spleen:* enlargement, splenic rub
- *Joints:* swelling, deformities
- *Neurologic:* abnormal mental state, cranial nerve abnormalities, peripheral nerve abnormalities, spinal cord signs

LABORATORY EVALUATION

The blood should be evaluated, both quantitatively and qualitatively. This is usually achieved

using automated equipment.

- Normal blood cell values are presented in **Table 1–4**. Normal total leukocyte and differential leukocyte counts are presented in **Table 1–5**.
- Hemoglobin concentration and red cell count are measured directly by automated instruments.
- Packed cell volume (*hematocrit*) is derived from the product of erythrocyte count and the mean red cell volume. It may also be measured directly by high-speed centrifugation of anticoagulated blood.
- Both the hemoglobin and the hematocrit are based on whole blood and are, therefore, dependent on plasma volume. If a patient is severely dehydrated, the hemoglobin and hematocrit will appear higher than if the patient were normovolemic; if the patient is fluid overloaded, those values will be lower than their actual level when normovolemic.
- Mean (red) cell volume (MCV), mean (red) cell hemoglobin (MCH), and mean (red) cell hemoglobin concentration (MCHC) are determined directly in automated cell analyzers. They may also be calculated by using the following formulas:

$$\text{MCV} = \frac{\text{hematocrit (mL/dL or \%)}}{\text{erythrocyte count } (\times 10^{12}/\text{L})} \times 10$$

- The units are femtoliters (fL).
- Mean cell hemoglobin (MCH) is calculated as follows:

$$\text{MCH} = \frac{\text{Hb (g/L)}}{\text{erythrocyte count } (\times 10^{12}/\text{L})} \times 10$$

- The units are picograms (pg) per cell.
- Mean corpuscular hemoglobin concentration (MCHC) is calculated as follows:

$$\text{MCHC} = \frac{\text{hemoglobin (g/L)}}{\text{hematocrit (mL/dL or \%)}} \times 10$$

- The units are grams of hemoglobin per deciliter (g/dL) of erythrocytes, or a percentage.
- The MCH may decrease or increase as a reflection of decreases or increases in red cell volume as well as actual increases or decreases in red cell hemoglobin concentration. The MCHC controls for those changes in red cell size, providing a more reliable measurement of hemoglobin concentration of red cells.
- Red cell distribution width (RDW) is calculated by automatic counters and reflects the variability in red cell size. The term “width” in RDW is misleading; it is a measure of the coefficient of variation of the volume of the red cells, and not the diameter. It is expressed as a percent.

$$\text{RDW} = (\text{Standard deviation of MCV} \div \text{mean MCV}) \times 100$$

  — Normal values are 11% to 14% of 1.0.
  — The presence of anisocytosis may be inferred from an elevated RDW value.
- Reticulocyte index. This variable is derived from the reticulocyte count and gives an estimate of the marrow response to anemia reflecting the red cell production rate.
  — The normal marrow with adequate iron availability can increase red cell production two to three times acutely and four to six times over a longer period of time.

— The reticulocyte index is used to determine if anemia is more likely the result of decreased red cell production or accelerated destruction in the circulation (hemolysis).
— By convention, hemolysis should be considered if the reticulocyte index is more than two times the basal value of 1.0.
— This calculation assumes (1) the red cell life span is ~100 days; (2) a normal reticulocyte is identifiable in the blood with supravital staining for 1 day; (3) the red cell life span is finite and the oldest 1% of red cells are removed and replaced each day; and (4) a reticulocyte count of 1% in an individual with a normal red cell count represents the normal red cell production rate per day thus, 1 is the basal reticulocyte index.
— The reticulocyte index provides the incidence of new red cells released per day as an estimate of marrow response to anemia.

- Consider a patient with a red cell count of 2×10^{12}/L and a reticulocyte count of 15%. The reticulocyte index is calculated as follows:
  — Corrected reticulocyte percent = observed reticulocyte percent × observed red cell count/normal red cell count. Calculation for patient values in this example = 15 × 2.0/5.0 = 6. This adjustment corrects the percent of reticulocytes for the decreased red cells in an anemic person. This calculation provides the prevalence of reticulocytes, but we want to know the incidence of reticulocytes (per day).
  — In anemia, under the influence of elevated erythropoietin, reticulocytes do not mature in the marrow for 3 days and then circulate for 1 day before they degrade their ribosomes and cannot be identified as such. Reticulocytes are released prematurely and thus may be identifiable in the circulation for 2 or 3 days and not reflect new red cells delivered that day, as in the normal state.
  — The corrected reticulocyte percent must be adjusted for premature release of reticulocytes. This is done by dividing the corrected reticulocyte percent by a factor related to the severity of anemia from 1.5 to 3. In practice, the value 2 is usually used as an approximation.
  — Thus, the corrected reticulocyte percent of 6 ÷ 2 results in a reticulocyte index of three times the basal value, indicating the anemia is hemolytic.
- Enumeration of erythrocytes, leukocytes, and platelets can be performed by manual methods by using diluting pipettes, a specially designed counting chamber, and a light microscope, but an electronic method provides much more precise data and is now used nearly universally for blood cell counts.
- Leukocyte differential count can be obtained from stained blood films prepared on glass slides. Automated techniques may be used for screening purposes, in which case abnormal cells are called out and examined microscopically by an experienced observer. Normal values for specific leukocyte types in adults are given in **Table 1–5**. The identifying features of the various types of normal leukocytes are shown in **Figure 1–1** and are detailed in *Williams Hematology*, 9th ed, Chap. 2; Chap. 60; Chap. 67; Chap. 73.
- Electronic methods that provide rapid and accurate classification of leukocyte types based largely on the physical properties of the cells have been developed and are in general use as described in *Williams Hematology*, 9th ed, Chap. 2.
- Properly stained blood films also provide important information on the morphology of erythrocytes and platelets as well as leukocytes.

- Examination of the blood film may reflect the presence of a number of diseases of the blood. These are listed in **Table 1–6**.

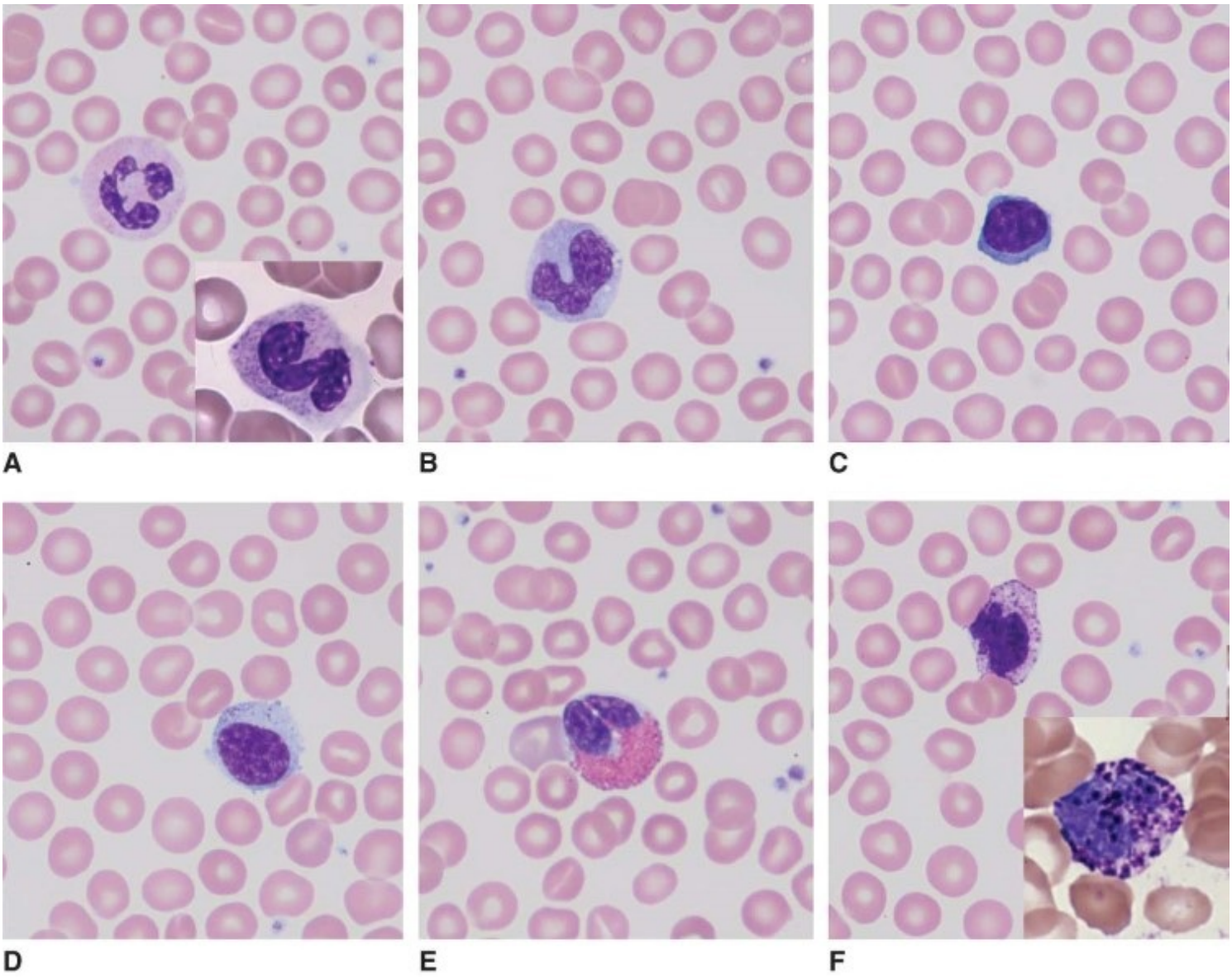


FIGURE 1–1 Images from a normal blood film showing major leukocyte types. The red cells are normocytic (normal size) and normochromic (normal hemoglobin content) with normal shape. The scattered platelets are normal in frequency and morphology. Images are taken from the optimal portion of the blood film for morphologic analysis. **A.** A platelet caught sitting in the biconcavity of the red cell in the preparation of the blood film—a segmented (polymorphonuclear) neutrophil and in the inset, a band neutrophil. This normal finding should not be mistaken for a red cell inclusion. **B.** A monocyte. **C.** A small lymphocyte. **D.** A large granular lymphocyte. Note that it is larger than the lymphocyte in **C** with an increased amount of cytoplasm containing scattered eosinophilic granules. **E.** An eosinophil. Virtually all normal blood eosinophils are bilobed and filled with relatively large (compared to the neutrophil) eosinophilic granules. **F.** Basophil and in inset a basophil that was less degranulated during film preparation, showing relatively large basophilic granules. The eosinophilic and basophilic granules are readily resolvable by light microscopy (×1000), whereas the neutrophilic granules are not resolvable, but in the aggregate impart a faint tan coloration to the neutrophil cytoplasm, quite distinctly different from the blue-gray cytoplasmic coloring of the monocyte and lymphocyte. (Source: *Williams Hematology*, 9th ed, Chap. 2, Figure 2–4.)

TABLE 1–4 BLOOD CELL VALUES IN A NORMAL POPULATION

	Men	Women	Either
White cell count,* × 10^9/L blood			7.8 (4.4–11.3)+
Red cell count, × 10^{12}/L blood	5.21 (4.52–5.90)	4.60 (4.10–5.10)	

Hemoglobin, g/dL blood	15.7 (14.0–17.5)[++]	13.8 (12.3–15.3)[++]	
Hematocrit (Volume of packed red cells as a ratio to a volume of blood)	0.46 (0.42–0.50)	0.40 (0.36–0.45)	
Mean cell volume, fL/red cell			88.0 (80.0–96.1)
Mean cell hemoglobin, pg/red cell			30.4 (27.5–33.2)
Mean cell hemoglobin concentration, g/dL red cell			34.4 (33.4–35.5)
Red cell distribution width, CV (%)			13.1 (11.5–14.5)
Platelet count, $\times 10^9$/L blood			311 (172–450)

*The International Committee for Standardization in Hematology recommends that SI units be used as follows: white cell count, number $\times 10^9$/L; red cell count, number $\times 10^{12}$/L; and hemoglobin, g/dL (dL = deciliter). The hematocrit (packed cell volume) is given as a numerical proportion, for example, 0.41, without designated units. Units of liter (of red cells) per liter (of blood) are implied. Mean cell volume is given as femtoliters (fL), mean cell hemoglobin as picograms (pg), and mean corpuscular hemoglobin concentration as g/dL. Platelets are reported as number $\times 10^9$/L. CV = coefficient of variation.

[+]The mean and reference intervals (normal range) are given. Because the distribution curves may be non-Gaussian, the reference interval is the nonparametric central 95% confidence interval. Results are based on 426 normal adult men and 212 normal adult women, with studies performed on the Coulter Model S-Plus IV. The normal intervals in this table may vary somewhat in different laboratories and in populations with varying ethnic distributions. For example, the mean neutrophil count in persons of African descent is approximately 1.5×10^9/L below that for individuals of European descent of similar sex and age. This difference also decreases the total leukocyte count in Americans of African descent by a similar concentration.

[++]The hemoglobin level of individuals of African descent is approximately 1.0 g/dL below that for individuals of European descent of similar sex and age.

TABLE 1–5 REFERENCE RANGES FOR LEUKOCYTE COUNT, DIFFERENTIAL COUNT, AND HEMOGLOBIN CONCENTRATION IN CHILDREN*

Age	Leukocytes Total ($\times 10^9$/L)	Neutrophils			Eosinophils	Basophils	Lymphocytes	Monocytes	Hemoglobin g/dL Blood
		Total	Band	Segmented					
12 mo	11.4 (6.0–17.5)	3.5 (1.5–8.5)	0.35 (0–1.0)	3.2 (1.0–8.5)	0.30 (0.05–0.70)	0.05 (0–0.20)	7.0 (4.0–10.5)	0.55 (0.05–1.1)	12.6 (11.1–14.1)
		31	*3.1*	*28*	*2.6*	*0.4*	*61*	*4.8*	
4 y	9.1 (5.5–15.5)	3.8 (1.5–8.5)	0.27 (0–1.0)	3.5 (1.5–7.5)	0.25 (0.02–0.65)	0.05 (0–0.2)	4.5 (2.0–8.0)	0.45 (0–0.8)	12.7 (11.2–14.3)
		42	*3.0*	*39*	*2.8*	*0.6*	*50*	*5.0*	
6 y	8.5 (5.0–14.5)	4.3 (1.5–8.0)	0.25 (0–1.0)	4.0 (1.5–7.0)	0.23 (0–0.65)	0.05 (0–0.2)	3.5 (1.5–7.0)	0.40 (0–0.8)	13.0 (11.4–14.5)
		51	*3.0*	*48*	*2.7*	*0.6*	*42*	*4.7*	
10 y	8.1 (4.5–13.5)	4.4 (1.8–8.0)	0.24 (0–1.0)	4.2 (1.8–7.0)	0.20 (0–0.60)	0.04 (0–0.2)	3.1 (1.5–6.5)	0.35 (0–0.8)	13.4 (11.8–15.0)
		54	*3.0*	*51*	*2.4*	*0.5*	*38*	*4.3*	
21 y	7.4 (4.5–11.0)	4.4 (1.8–7.7)	0.22 (0–0.7)	4.2 (1.8–7.0)	0.20 (0–0.45)	0.04 (0–0.2)	2.5 (1.0–4.8)	0.30 (0–0.8)	M: 15.5 (13.5–17.5)
		59	*3.0*	*56*	*2.7*	*0.5*	*34*	*4.0*	F: 13.8 (12.0–15.6)

*The means and ranges are in $\times 10^9$ cells per L. This table is provided as a guide. Normal ranges should be validated by the clinical laboratory for the specific methods in use. The number in *italics* is mean percentage of total leukocytes.
Source: *Williams Hematology*, 8th ed, Chap. 1, Table 1–5.

TABLE 1–6 DISEASES IN WHICH EXAMINATION OF THE BLOOD FILM CAN SUGGEST OR CONFIRM THE DISORDER

Disease	Findings on Blood Film
Immune hemolytic anemia	Spherocytes, polychromatophilia, erythrocyte agglutination, erythrophagocytosis
Hereditary spherocytosis	Spherocytes, polychromatophilia
Hereditary elliptocytosis	Elliptocytes
Hereditary ovalocytosis	Ovalocytes
Hemoglobin C disease	Target cells, spherocytes
Hemoglobin S disease	Sickle cells

Hemoglobin SC	Target cells, sickle cells
Thalassemia minor (alpha or beta)	Microcytosis, target cells, teardrop cells, basophilic stippling, other misshapen cells
Thalassemia major (alpha or beta)	Microcytosis, target cells, basophilic stippling, teardrop cells, other misshapen cells (often more exaggerated than minor form)
Iron deficiency	Microcytosis, hypochromia, absence of basophilic stippling
Lead poisoning	Basophilic stippling
Vitamin B_{12} or folic acid deficiency	Macrocytosis, with oval macrocytes, hypersegmented neutrophils
Myeloma, macroglobulinemia	Pathologic rouleaux formation
Malaria, babesiosis, others	Parasites in the erythrocytes
Consumptive coagulopathy	Fragmented red cells (schistocytes)
Mechanical hemolysis	Fragmented red cells (schistocytes)
Severe infection	Increase in neutrophils; increased band forms, Döhle bodies, neutrophil vacuoles
Infectious mononucleosis	Reactive lymphocytes
Agranulocytosis	Decreased neutrophils
Allergic reactions	Eosinophilia
Chronic lymphocytic leukemia	Absolute small-cell lymphocytosis
Chronic myelogenous leukemia	Promyelocytes, myelocytes, basophils, hypersegmented neutrophils
Oligoblastic myelogenous leukemia (refractory anemia with excess blast cells, myelodysplasia)	Blast forms, acquired Pelger-Huët neutrophil nuclear abnormality, anisocytosis, poikilocytosis, abnormal platelets
Clonal cytopenias (myelodysplasia)	Anisocytosis, anisochromia, poikilocytosis, hypogranular neutrophils, acquired Pelger-Huët neutrophil nuclear abnormality, neutropenia, thrombocytopenia, giant platelets
Acute leukemia	Blast cells
Thrombocytopenia	Decreased platelets
Thrombocytosis or thrombocythemia	Increased platelets

Infancy and Childhood

- Some components of the blood count in infancy and childhood differ significantly from those in adults.
- Hemoglobin levels are high at birth (19.3 ± 2.2 [s.d.] g/dL) but fall over the first 12 weeks of life to reach levels that persist throughout childhood (11.3 ± 0.9 [s.d.] g/dL). Adult levels in males are achieved after puberty. Red cell values for infants during the first 12 weeks of life are given in *Williams Hematology*, 9th ed, Chap. 7, **Table 7–2**.
- The mean leukocyte count is high at birth (mean of 18 × 10^9/L); neutrophils comprise about 60% of the cells. The leukocyte count falls over the next 2 weeks or so, to reach levels that persist throughout childhood. Lymphocytes are the predominant cell type for the remainder of the first 4 years of life (45%–55%). Further details can be found in *Williams Hematology*, 9th ed, Chap. 7, **Table 7–3**.
- Platelet counts are at adult levels throughout childhood.
- Leukocyte function may be depressed in normal infants in the newborn period.
- Reference values for coagulation factors in neonates and infants may be found in *Williams Hematology*, 9th ed, Chap. 7, **Table 7–6**, and for coagulation factor inhibitors in neonates and

infants in **Table 7–7**.

Effects of Aging

- See *Williams Hematology*, 9th ed, Chap. 9.
- Blood count and cell function may also vary with advanced age.
- The hemoglobin level of men older than 65 years of age is statistically lower than that of younger men, even in the absence of a demonstrable cause for anemia, but is not sufficiently lower to warrant use of specific normal values for older men. Anemia in an older person warrants careful evaluation as to its cause before concluding it is the anemia of aging.
- The hemoglobin level in women does not change significantly with advancing age.
- Total and differential leukocyte counts also do not change significantly with advancing age.
- Leukocytosis in response to infection (eg, appendicitis or pneumonia) is the same in older individuals as in people younger than age 60, but special studies indicate that the marrow granulocyte reserve may be reduced in older persons.
- Both cellular and humoral immune responses are reduced in older patients.
- The erythrocyte sedimentation rate increases significantly with age.
- Aging is associated with a net procoagulant propensity and an increased risk of venous thrombosis.

Utility of the Blood Film in Diagnosis

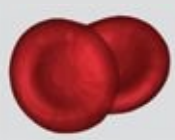
- The blood film is invaluable in developing the differential diagnosis or the specific diagnosis of a blood cell disorder. **Table 1–6** lists several situations in which the blood film can be important or decisive.

The Marrow

- Examination of the marrow is important in the diagnosis and management of a variety of hematologic disorders.
- All bones contain hematopoietic marrow at birth.
- Fat cells begin to replace hematopoietic marrow in the extremities in the fifth to sixth year of life.
- In adults, hematopoietic marrow is principally located in the axial skeleton (ribs, spine, sternum, pelvis, scapula, clavicle, and base of the skull) and the proximal quarter of the humeri and femora.
- Hematopoietic marrow cellularity is reduced in the elderly, falling after age 60 from about 50% to 30%, roughly in inverse proportion to age.
- Marrow is obtained by aspiration and/or needle biopsy. The most frequently utilized site is the iliac crest at the posterior superior iliac spine. Modern biopsy instruments provide excellent material for diagnostic study.
- Aspirated marrow may be evaluated after preparation of films on glass slides and appropriate staining.
- Marrow biopsies are examined after fixation, sectioning, and staining. “Touch” preparations made by holding the biopsy specimen with a forceps and touching the end to one or more clean slides in several places. Imprints of the marrow remain on the slide. The slides are quickly air

dried, fixed with methanol, and stained. Morphologic details of the cells are preserved with this type of preparation and thus provide additional information.

- Interpretation of marrow films and biopsy sections is discussed in *Williams Hematology*, 9th ed, Chap. 3 and in chapters describing specific diseases for which a marrow is usually performed. *Williams Hematology*, 9th ed, **Table 3–1** contains the normal differential count of cells in the marrow.



For a more detailed discussion, see Marshall A. Lichtman and Linda J. Burns: Approach to the Patient, Chap. 1; Daniel H. Ryan: Examination of Blood Cells, Chap. 2; Daniel H. Ryan: Examination of the Marrow, Chap. 3; James Palis and George B. Segel: Hematology of the Fetus and Newborn, Chap. 7; William B. Ershler, Andrew S. Artz, and Bindu Kanapuru: Hematology in Older Persons, Chap. 9; C. Wayne Smith: Morphology of Neutrophils, Eosinophils, and Basophils, Chap. 60; Steven D. Douglas, Ann G. Douglas: Morphology of Monocytes and Macrophages, Chap. 67; Natarajan Muthusamy and Michael A. Caligiuri: Structure of Lymphocytes and Plasma Cells, Chap. 73 in *Williams Hematology*, 9th ed.

PART II

DISORDERS OF RED CELLS

CHAPTER 2
Classification of Anemias and Polycythemias

- Clinically significant red cell disorders can be classified into:
  — Disorders in which the red cell mass is decreased (anemias). The principal effect is decreased oxygen-carrying capacity of the blood. Their severity is best expressed in terms of hemoglobin concentration.
  — Disorders in which the red cell mass is increased (polycythemias also known as erythrocytoses). The principal effect is related to an increased viscosity of the blood (see **Figure 2–1**). In addition to their specific effects, they are best expressed in terms of packed red cell volume (hematocrit).
- The red cell mass is the volume of the mass of red cells in the circulation.
  — The normal red cell mass among women is 23 to 29 mL/kg.
  — The normal red cell mass among men is 26 to 32 mL/kg.
  — More accurate formulas based on body surface have been recommended.
- Because the red cells are measured either as a concentration in the blood as the red cell count, the hemoglobin content of the blood, or the hematocrit (packed red cell volume per 100 mL of blood), rather than the volume of the red cell mass in the total circulation, the anemias and polycythemias can each be subclassified as:
  — Relative, where the red cell mass is normal but the amount of plasma is increased (relative anemia) or decreased (polycythemia)
  — Absolute, where the red cell mass is decreased (true anemia) or increased (true polycythemia)
- The various types of anemia are classified in **Table 2–1**.
- It is essential that the specific cause of anemia be determined. The initial laboratory approach to the diagnosis of anemia follows, and these four studies should be the prelude to guide further specific testing.
  — Hematocrit, hemoglobin, or red cell count to determine degree of anemia. In most cases, these three variables are closely correlated. Hemoglobin concentration is the most direct measure of oxygen-carrying capacity.
  — Red cell indices, such as mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) to determine whether normocytic, macrocytic, or microcytic and normochromic or hypochromic red cells are present on average
  — Measurement of red cell distribution width (RDW) to obtain a measure of anisocytosis
  — Reticulocyte count or index to estimate whether marrow response suggests inadequacy of red cell production or an appropriate erythropoietic response to hemolysis (or acute bleeding). The latter is usually readily apparent clinically.
  — Examination of the blood film to determine red cell size and shape, hemoglobin content,

presence of red cell inclusions, presence of agglutination or rouleaux formation, nonhematopoietic particles such as parasites (ie, *Babesia* and *Plasmodium* species) and helminths (ie, *Wuchereria bancrofti*, nematodes), and accompanying abnormalities of white cells and platelets

- Important caveats:
  - — Red cell size and hemoglobin content are best determined from their indices because the blood film will usually make evident only gross deviations (eg, the need to estimate red cell volume from a two-dimensional area). Moreover, the blood in macrocytic anemia usually contains many microcytic cells and in microcytic anemias, many normocytic cells, which make determination of the average red cell volume from a blood film difficult.
  - — In general, the abnormalities in size, hemoglobin content, and shape are approximately correlated with severity of anemia. If the anemia is slight, the other changes are often subtle.
  - — Anemia classically categorized as macrocytic or microcytic may be present in the face of red cell volumes that are in the normal range. This may be the case because the anemia is so mild that red cell volumes have not yet deviated beyond the normal range, or may be the case with more severe anemias because of confounding effects of two causal factors (eg, iron deficiency and folate deficiency), or well-established megaloblastic anemia may have normocytic index in otherwise asymptomatic individuals such as those being silent carriers or having alpha thalassemia trait (one or two alpha globin deletions) (see Chap. 15).
- A classification of the major causes of polycythemia is shown in **Table 2–2**.
- It is important to search for the specific cause of polycythemia. The diagnosis of polycythemias is discussed in Chaps. 27 (polyclonal polycythemias) and 41 (polycythemia vera).

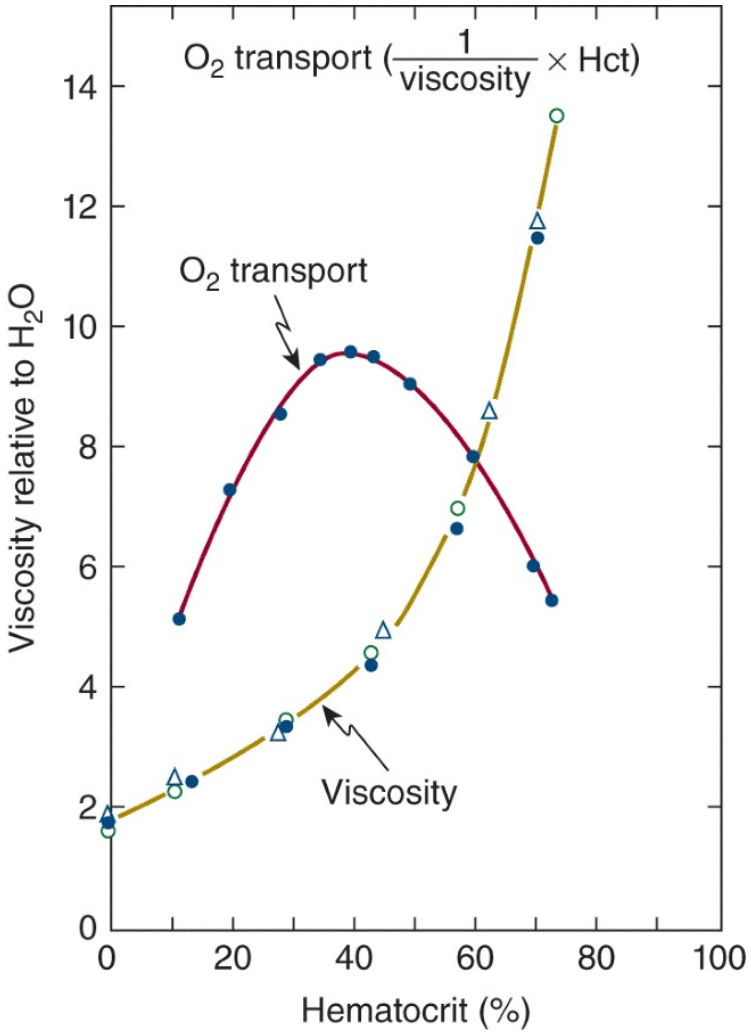


FIGURE 2–1 Viscosity of heparinized normal human blood related to hematocrit (Hct). Viscosity is measured with an Ostwald viscosimeter at 37°C and expressed in relation to viscosity of saline solution. Oxygen transport is computed from Hct and oxygen flow (1/viscosity) and is recorded in arbitrary units. Please note this curve of oxygen transport applies when red cell mass is normal. When red cell mass is increased the curve shifts to the right, when decreased it shifts to the left.

TABLE 2–1 CLASSIFICATION OF ANEMIA

I. Absolute anemia (decreased red cell volume)
- A. Decreased red cell production
  - 1. Acquired
    - a. Pluripotential stem cell failure
      - (1) Autoimmune (aplastic anemia) (see Chap. 3)
        - (a) Radiation induced
        - (b) Drugs and chemicals (chloramphenicol, benzene, etc.)
        - (c) Viruses (non-A-G, H hepatitis, Epstein-Barr virus, etc.)
        - (d) Idiopathic
      - (2) Anemia of leukemia and of myelodysplastic syndromes (see Chaps. 44 and 45)
      - (3) Anemia associated with marrow infiltration (see Chap. 12)
      - (4) Postchemotherapy (see Chap. 38)
    - b. Erythroid progenitor cell failure
      - (1) Pure red cell aplasia (parvovirus B19 infection, drugs, associated with thymoma, autoantibodies, etc. [see Chap. 4])
      - (2) Endocrine disorders (see Chap. 6)
      - (3) Acquired sideroblastic anemia (drugs, copper deficiency, etc. [see Chap. 11])
    - c. Functional impairment of erythroid and other progenitors due to nutritional and other causes
      - (1) Megaloblastic anemias (see Chap. 8)
        - (a) B_{12} deficiency

(b) Folate deficiency
(c) Acute megaloblastic anemia because of nitrous oxide (N_2O)
(d) Drug-induced megaloblastic anemia (pemetrexed, methotrexate, phenytoin toxicity, etc.)
(2) Iron-deficiency anemia (see Chap. 9)
(3) Anemia resulting from other nutritional deficiencies (see Chap. 10)
(4) Anemia of chronic disease and inflammation (see Chap. 5)
(5) Anemia of renal disease (see Chap. 5)
(6) Anemia caused by chemical agents (lead toxicity [see Chap. 20])
(7) Acquired thalassemias (seen in some clonal hematopoietic disorders [see Chaps. 15 and 40])
(8) Erythropoietin antibodies (see Chap. 4)

2. Hereditary
a. Pluripotential hematopoietic stem cell failure (see Chap. 3)
(1) Fanconi anemia
(2) Shwachman syndrome
(3) Dyskeratosis congenita
b. Erythroid progenitor cell failure
(1) Diamond-Blackfan syndrome (see Chap. 3)
(2) Congenital dyserythropoietic syndromes (see Chap. 7)
c. Functional impairment of erythroid and other progenitors from nutritional and other causes
(1) Megaloblastic anemias (see Chap. 8)
(a) Selective malabsorption of vitamin B_{12} (Imerslund-Gräsbeck disease)
(b) Congenital intrinsic factor deficiency
(c) Transcobalamin II deficiency
(d) Inborn errors of cobalamin metabolism (methylmalonic aciduria, homocystinuria, etc.)
(e) Inborn errors of folate metabolism (congenital folate malabsorption, dihydrofolate deficiency, methyltransferase deficiency, etc.)
(2) Inborn purine and pyrimidine metabolism defects (Lesch-Nyhan syndrome, hereditary orotic aciduria, etc.)
(3) Disorders of iron metabolism (see Chap. 9)
(a) Hereditary atransferrinemia
(b) Hypochromic anemia caused by divalent metal transporter (DMT)-1 mutation
(4) Hereditary sideroblastic anemia (see Chap. 11)
(5) Thalassemias (see Chap. 15)

B. Increased red cell destruction
1. Acquired
a. Mechanical
(1) Macroangiopathic (march hemoglobinuria, artificial heart valves [see Chap. 19])
(2) Microangiopathic (disseminated intravascular coagulation [DIC]; thrombotic thrombocytopenic purpura [TTP]; vasculitis [see Chaps. 19, 85, and 90])
(3) Parasites and microorganisms (malaria, bartonellosis, babesiosis, *Clostridium perfringens*, etc. [see Chap. 21])
b. Antibody mediated
(1) Warm-type autoimmune hemolytic anemia (see Chap. 22)
(2) Cryopathic syndromes (cold agglutinin disease, paroxysmal cold hemoglobinuria, cryoglobulinemia [see Chap. 23])
(3) Transfusion reactions (immediate and delayed [see Chap. 91])
c. Hypersplenism (see Chap. 26)
d. Red cell membrane disorders (see Chap. 13)
(1) Spur cell hemolysis
(2) Acquired acanthocytosis and acquired stomatocytosis, etc.
e. Chemical injury and complex chemicals (arsenic, copper, chlorate, spider, scorpion, and snake venoms, etc. [see Chap. 20])
f. Physical injury (heat, oxygen, radiation [see Chap. 20])

2. Hereditary
a. Hemoglobinopathies (see Chap. 16)
(1) Sickle cell disease
(2) Unstable hemoglobins
b. Red cell membrane disorders (see Chap. 13)
(1) Cytoskeletal membrane disorders (hereditary spherocytosis, elliptocytosis, pyropoikilocytosis)

(2) Lipid membrane disorders (hereditary abetalipoproteinemia, hereditary stomatocytosis, etc.)
(3) Membrane disorders associated with abnormalities of erythrocyte antigens (McLeod syndrome, Rh deficiency syndromes, etc.)
(4) Membrane disorders associated with abnormal transport (hereditary xerocytosis)
c. Red cell enzyme defects (pyruvate kinase, 5' nucleotidase, glucose-6-phosphate dehydrogenase deficiencies, other red cell membrane disorders [see Chap. 14])
d. Porphyrias (congenital erythropoietic and hepatoerythropoietic porphyrias, rarely congenital erythropoietic protoporphyria [see Chap. 28])

C. Blood loss and blood redistribution
1. Acute blood loss
2. Splenic sequestration crisis (see Chap. 26)

II. Relative (increased plasma volume)
A. Macroglobulinemia (see Chap. 69)
B. Pregnancy
C. Athletes (see Chap. 19)
D. Postflight astronauts

Source: *Williams Hematology*, 9th ed, Chap. 34, Table 34–1.

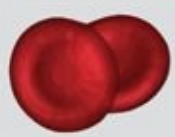
TABLE 2–2 CLASSIFICATION OF POLYCYTHEMIA

I. **Absolute (true) polycythemia (increased red cell volume) (see Chap. 27)**
A. Primary polycythemia
1. Acquired: polycythemia vera (see Chap. 41)
2. Hereditary (see Chap. 27): primary familial and congenital polycythemia (PFCP)
a. Erythropoietin receptor mutations
b. Unknown gene mutations
B. Secondary polycythemia
1. Acquired (see Chap. 27)
a. Hypoxemia
(1) Chronic lung disease
(2) Sleep apnea
(3) Right-to-left cardiac shunts
(4) High altitude
(5) Smoking
b. Carboxyhemoglobinemia (see Chap. 18)
(1) Smoking
(2) Carbon monoxide poisoning
c. Autonomous erythropoietin production (see Chap. 27)
(1) Hepatocellular carcinoma
(2) Renal cell carcinoma
(3) Cerebellar hemangioblastoma
(4) Pheochromocytoma
(5) Parathyroid carcinoma
(6) Meningioma
(7) Uterine leiomyoma
(8) Polycystic kidney disease
d. Exogenous erythropoietin administration ("EPO doping") (see Chap. 27)
e. Complex or uncertain etiology
(1) Postrenal transplant (probable abnormal angiotensin II signaling) (see Chap. 27)
(2) Androgen/anabolic steroids (see Chap. 27)
2. Hereditary
a. High-oxygen affinity hemoglobins (see Chap. 17)
b. 2,3-Bisphosphoglycerate deficiency (see Chap. 14)
c. Congenital methemoglobinemias (recessive, ie, cytochrome b5 reductase deficiency, dominant globin mutations [see Chaps. 17 and 27])
C. Disorders of Hypoxia sensing (see Chap. 27)
1. Proven or suspected congenital disorders of hypoxia sensing
a. Chuvash polycythemia

b. High erythropoietin polycythemias due to mutations of von Hippel-Lindau gene other than Chuvash mutation
c. HIF-2 α (*EPASI*) mutations
d. PHD2 (*EGLN1*) mutations

II. **Relative (spurious) polycythemia (normal red cell volume) (see Chap. 27)**
A. Dehydration
B. Diuretics
C. Smoking
D. Gaisböck syndrome

Source: *Williams Hematology*, 9th ed, Chap. 34, Table 34–2.



For a more detailed discussion, see Josef T. Prchal: Clinical Manifestations and Classification of Erythrocyte Disorders, Chap. 34; Josef T. Prchal: Erythropoiesis, Chap. 32; Josef T. Prchal: Primary and Secondary Polycythemia (Erythrocytosis), Chap. 57; Mohandas Narla: Structure and Composition of the Erythrocyte, Chap. 31 in *Williams Hematology*, 9th ed.

CHAPTER 3
Aplastic Anemia: Acquired and Inherited

DEFINITION

- Aplastic anemia is marked by pancytopenia with markedly hypocellular marrow and normal marrow cell cytogenetics.
- Incidence worldwide is two to five cases/million population per year and five to twelve cases/million population per year in the United States (and in other industrialized countries). Incidence is approximately twice as high in Asian countries.
- Peak incidence is between ages 15 and 25 and 65 and 69 years.
- The definitions for spectrum of severity of aplastic anemia are shown in Table 3–1.

TABLE 3–1 DEGREE OF SEVERITY OF ACQUIRED APLASTIC ANEMIA*

Diagnostic Categories	Hemoglobin	Reticulocyte Concentration	Neutrophil Count	Platelet Count	Marrow Biopsy	Comments
Moderately severe	< 100 g/L	< 40 × 10^9/L	< 1.5 × 10^9/L	< 50 × 10^9/L	Marked decrease of hematopoietic cells	At the time of diagnosis at least two of three blood counts should meet these criteria.
Severe	< 90 g/L	< 30.0 × 10^9/L	< 0.5 × 10^9/L	< 30.0 × 10^9/L	Marked decrease or absence of hematopoietic cells	Search for a histocompatible sibling should be made if age permits.
Very severe	< 80 g/L	< 20.0 × 10^9/L	< 0.2 × 10^9/L	< 20.0 × 10^9/L	Marked decrease or absence of hematopoietic cells	Search for a histocompatible sibling should be made if age permits.

*These values are approximations and must be considered in the context of an individual patient's situation. (In some clinical trials, the blood count thresholds for moderately severe aplastic anemia are higher [eg, platelet count < 100 × 10^9/L and absolute reticulocyte count < 60,000 × 10^9/L].) The marrow biopsy may contain the usual number of lymphocytes and plasma cells; "hot spots," focal areas of erythroid cells, may be seen. No fibrosis, abnormal cells, or malignant cells should be evident in the marrow. Dysmorphic features of blood or marrow cells are not features of acquired aplastic anemia. Ethnic differences in the lower limit of the absolute neutrophil count should be considered. (See *Williams Hematology*, 9th ed, Chaps. 64 and 65.)
Source: *Williams Hematology*, 9th ed, Chap. 35, Table 35–1.

ETIOLOGY AND PATHOGENESIS

Pathogenesis

- Immune suppression of marrow by autoreactive T lymphocytes
- Toxic injury to stem and/or progenitor cells (eg, certain chemotherapy or drugs) (see Table 3–2)
- Inherited intrinsic stem cell defect (eg, Fanconi anemia)

TABLE 3–2	SOME DRUGS ASSOCIATED WITH MODERATE RISK OF APLASTIC ANEMIA*
Acetazolamide	
Carbamazepine	
Chloramphenicol	
Gold salts	
Hydantoins	
Oxyphenbutazone	
Penicillamine	
Phenylbutazone	
Quinacrine	

*Drugs with 30 or more reported cases.
Source: *Williams Hematology*, 9th ed, Chap. 35, Table 35–3.

Acquired (see Table 3–3)

TABLE 3–3	ETIOLOGIC CLASSIFICATION OF APLASTIC ANEMIA
ACQUIRED	
Autoimmune	
Drugs (see Table 3–2)	
Toxins	
Benzene	
Chlorinated hydrocarbons	
Organophosphates	
Viruses	
Epstein-Barr virus	
Non-A, -B, -C, -D, -E, or -G hepatitis virus	
Human immunodeficiency virus (HIV)	
Paroxysmal nocturnal hemoglobinuria	
Autoimmune/connective tissue disorders	
Eosinophilic fasciitis	
Immune thyroid disease (Graves disease, Hashimoto thyroiditis)	
Rheumatoid arthritis	
Systemic lupus erythematosus	
Thymoma	
Pregnancy	
Iatrogenic	
Radiation	
Cytotoxic drug therapy	
INHERITED	
Fanconi anemia	
Dyskeratosis congenita	
Shwachman-Diamond syndrome	
Other rare syndromes (see Table 3–4)	

Source: *Williams Hematology*, 9th ed, Chap. 35, Table 35–2.

- Acquired T lymphocyte–mediated autoimmune suppression of hematopoietic stem cells and/or progenitor cells in most cases (~70%)
- Paroxysmal nocturnal hemoglobinuria (PNH) (may be manifest by cytopenias and hypoplastic marrow)

- Chemicals (eg, high-dose benzene exposure); rare today in countries with workplace and product regulations limiting exposure
- Drugs (eg, chloramphenicol; see **Table 3–2** for most frequent offenders; see also *Williams Hematology*, 9th ed, Chap. 35, **Table 35–3** for a more complete list)
- Viruses (eg, Epstein-Barr; non-A, -B, -C, -D, -E, or -G hepatitis; HIV)
- Immune and connective tissue diseases (eg, eosinophilic fasciitis, Hashimoto thyroiditis, Graves disease, systemic lupus erythematosus)
- Pregnancy
- Iatrogenic or accidental (eg, intensive radiation to marrow-bearing bones, intensive marrow-suppressive chemotherapy)

Inherited (see Table 3–3)

- Fanconi anemia
  - — Inheritance is autosomal recessive.
  - — Any of 16 gene mutations, *FANCA* through *FANCQ*, account for about 95% of cases.
  - — Macrocytosis and poikilocytosis may precede cytopenias.
  - — Cytopenias, sometimes starting with thrombocytopenia, develop after age 5 to 10 years.
  - — Marrow hypocellularity explains cytopenias.
  - — Short stature; abnormal skin pigmentation (café-au-lait spots); skeletal abnormalities (eg, dysplastic radii and thumbs); heart, kidney, and eye anomalies; microcephaly; and hypogonadism in different combinations are often noted.
  - — Chromosome fragility may be present, especially after exposure to DNA cross-linking agents such as diepoxybutane (used as a diagnostic test).
  - — Androgens occasionally may improve hematopoiesis.
  - — Allogeneic hematopoietic stem cell transplantation can be curative.
  - — There is risk of acute myelogenous leukemia and other cancers.
- Dyskeratosis congenita
  - — Inheritance patterns: autosomal dominant, autosomal recessive, and X-linked (see *Williams Hematology*, 9th ed, Chap. 35, **Table 35–10**)
  - — Gene mutations identified in majority of cases
  - — Mutations involving genes encoding proteins in the telomerase complex
  - — Resulting abnormalities in telomere length
  - — Mucocutaneous (eg, skin hyperpigmentation or hypopigmentation, alopecia leukoplakia) and finger and toenail abnormalities (ridging and longitudinal splitting, atrophy) in childhood
  - — Pulmonary (eg, fibrosis), gastrointestinal (eg, esophageal webs), urogenital (eg, hypospadias), neurologic (eg, learning impairment), skeletal (eg, hypoplasia of mandible) findings
  - — Aplastic anemia in early adulthood: principal cause of death
  - — Increased incidence of various mucosal cancers (eg, squamous cell carcinoma of mouth, nasopharynx, esophagus, rectum, vagina, others)
- Shwachman-Diamond syndrome
  - — The cause is mutation in the *SBDS* (Shwachman-Bodian-Diamond syndrome) gene on chromosome 7.

— Exocrine pancreatic insufficiency and neutropenia occur. Pancreatic endocrine function (insulin secretion) generally remains intact.
— Neutropenia with functionally abnormal neutrophils (defective chemotaxis) is present in virtually all patients.
— Anemia and thrombocytopenia are less common.
— Elevated hemoglobin F occurs in most patients.
— Pancytopenia occurs in about 20% of patients.
— Patients usually present in early infancy with malabsorption; steatorrhea; failure to thrive; and deficiencies of fat-soluble vitamins A, D, E, and K.
— Approximately 50% of patients regain exocrine pancreatic function during later childhood.
— Skeletal anomalies (eg, short stature, osteochondrodysplasia [cartilage and bone anomalies], osteoporosis) are present in about 75% of patients.
— Recurrent bacterial infections (eg, upper respiratory tract infections, otitis media, sinusitis, pneumonia, paronychia, osteomyelitis, bacteremia) occur.
— Enzyme replacement therapy is given for exocrine pancreatic insufficiency.
— Progression to multicytopenia, hypoplastic marrow, myelodysplasia, or acute myelogenous leukemia can occur.
— Allogeneic hematopoietic stem cell transplantation can be curative.

- Other rare causes of aplastic anemia are shown in **Table 3–4**

TABLE 3–4 OTHER RARE INHERITED SYNDROMES ASSOCIATED WITH APLASTIC ANEMIA

Disorder	Findings	Inheritance	Mutated Gene
Ataxia-pancytopenia (myelocerebellar disorder)	Cerebellar atrophy and ataxia; aplastic pancytopenia; ± monosomy 7; increased risk of AML	AD	Unknown
Congenital amegakaryocytic thrombocytopenia	Thrombocytopenia; absent or markedly decreased marrow megakaryocytes; hemorrhagic propensity; elevated thrombopoietin; propensity to progress to aplastic pancytopenia; propensity to evolve to clonal myeloid disease	AR (compound heterozygotes)	*MPL*
DNA ligase IV deficiency	Pre- and postnatal growth delay; dysmorphic facies; aplastic pancytopenia	AR	*LIG4*
Dubowitz syndrome	Intrauterine and postpartum growth failure; short stature; microcephaly; mental retardation; distinct dysmorphic facies; aplastic pancytopenia; increased risk of AML and ALL	AR	Unknown
Nijmegen breakage syndrome	Microcephaly; dystrophic facies; short stature; immunodeficiency; radiation sensitivity; aplastic pancytopenia; predisposition to lymphoid malignancy	AR	*NBS1*
Reticular dysgenesis (type of severe immunodeficiency syndrome)	Lymphopenia; anemia and neutropenia; corrected by hematopoietic stem cell transplantation	XLR	Unknown
Seckel syndrome	Intrauterine and postpartum growth failure; microcephaly; characteristic dysmorphic facies (bird-headed profile); aplastic pancytopenia; increased risk of AML	AR	*ATR* (and *RAD3*-related gene); *PCNT*
WT syndrome	Radial/ulnar abnormalities; aplastic pancytopenia;	AD	Unknown

	increased risk of AML

AD, autosomal dominant; ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; AR, autosomal recessive; XLR, X-linked recessive.

The listed clinical findings in each syndrome are not comprehensive. The designated clinical findings may not be present in all cases of the syndrome. Isolated cases of familial aplastic anemia with or without associated anomalies that are not consistent with Fanconi anemia or other defined syndromes have been reported.

Source: *Williams Hematology,* 9th ed, Chap. 35, Table 35–9.

CLINICAL FEATURES

- Fatigue, pallor, dyspnea on exertion, bleeding, or infections occur as a consequence of the cytopenias.
- Physical examination generally is unrevealing except for signs of anemia, bleeding, or infection.

LABORATORY FEATURES

- Pancytopenia is present.
- Red cells may be macrocytic.
- Marrow is markedly hypocellular (**Figure 3–1**).
- Abnormal clonal cytogenetic findings suggest hypoplastic myelodysplastic syndrome (clonal myeloid disease) rather than aplastic anemia.
- Blast cells in marrow suggest hypoplastic acute myelogenous leukemia.
- Presence of CD55,CD59 on blood cells by flow cytometry rules out PNH.

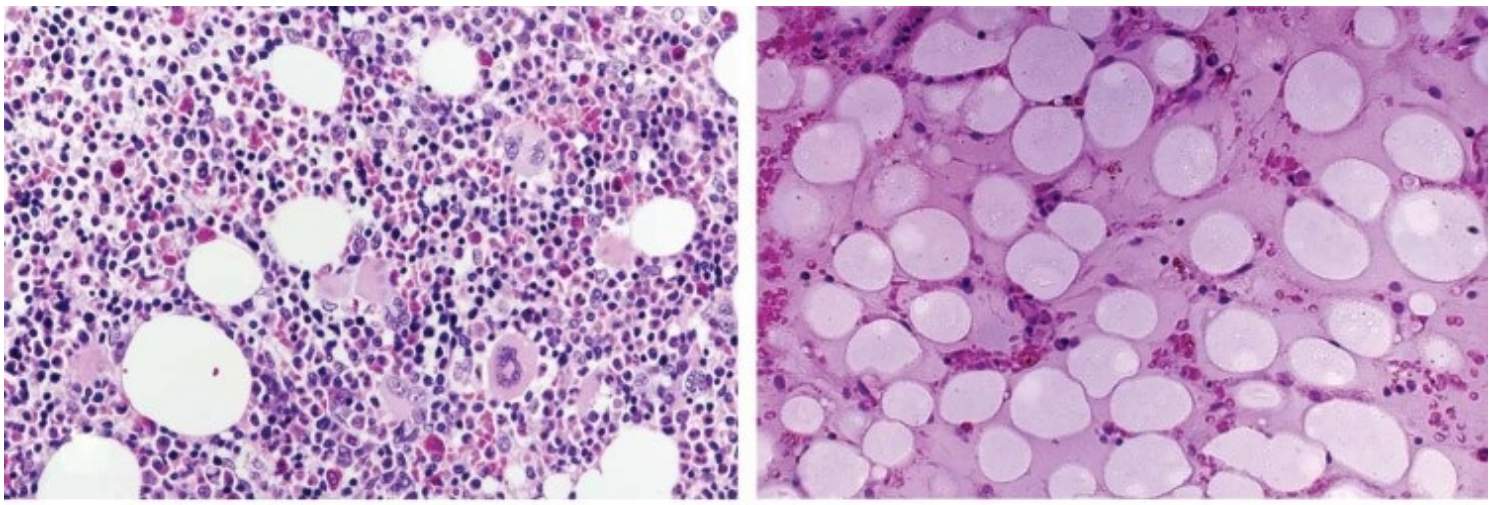


FIGURE 3–1 Marrow biopsy in aplastic anemia. **A.** Normal marrow biopsy section of a young adult. **B.** Marrow biopsy section of a young adult with very severe aplastic anemia. The specimen is devoid of hematopoietic cells and contains only scattered lymphocytes and stromal cells. The hematopoietic space is replaced by reticular cells (preadipocytic fibroblasts) converted to adipocytes. (Source: *Williams Hematology,* 9th ed, Fig. 35–2.)

Table 3–5 lists important diagnostic procedures.

TABLE 3–5	APPROACH TO DIAGNOSIS

- History and physical examination
- Complete blood counts, reticulocyte count, and examination of the blood film
- Marrow aspiration and biopsy
- Marrow cell cytogenetics to evaluate presence of a clonal myeloid disease
- DNA stability test as markers of Fanconi anemia
- Immunophenotyping of red and white cells, especially for CD55, CD59 to exclude paroxysmal nocturnal hemoglobinuria
- Direct and indirect antiglobulin (Coombs) test to rule out immune cytopenia
- Serum lactate dehydrogenase and uric acid, which if increased may reflect neoplastic cell turnover
- Liver function tests to assess evidence of any recent hepatitis virus exposure
- Screening tests for hepatitis viruses A, B, and C
- Screening tests for Epstein-Barr virus, cytomegalovirus, and HIV
- Serum B_{12} and red cell folic acid levels to rule out cryptic megaloblastic pancytopenia
- Serum iron, iron-binding capacity, and ferritin as a baseline prior to chronic transfusion therapy

Source: *Williams Hematology,* 9th ed, Chap. 35, Table 35–4.

TREATMENT

Table 3–6 lists important initial steps in management.

TABLE 3–6 INITIAL MANAGEMENT OF APLASTIC ANEMIA

- Discontinue any potential offending drug and use an alternative class of agents if essential.
- Anemia: transfuse leukocyte-depleted, irradiated red cells as required for very severe anemia.
- Very severe thrombocytopenia or thrombocytopenic bleeding: consider ∈-aminocaproic acid; transfusion of platelets as required; thrombopoietin receptor agonists under study.
- Severe neutropenia: use infection precautions.
- Fever (suspected infection): microbial cultures; broad-spectrum antibiotics if specific organism not identified, granulocyte colony-stimulating factor (G-CSF) in dire cases. If child or small adult with profound and prolonged infection (eg, gram-negative bacteria, fungus, persistent positive blood cultures) can consider neutrophil transfusion from a G-CSF pretreated donor.
- If transplant candidate, immediate assessment for allogeneic stem cell transplantation: histocompatibility testing of patient, parents, and siblings. Search databases for unrelated donor, if appropriate.

Source: *Williams Hematology,* 9th ed, Chap. 35, Table 35–5.

- Allogeneic hematopoietic stem cell transplantation is often curative.
  - — It is indicated in patients < 55 years with a suitable donor and without serious comorbid conditions. Age for transplantation may increase with advances in transplantation.
  - — Less than one third of patients in the United States have a matched sibling donor.
  - — Success of transplantation is a function of age and whether related donor is used (**Figure 3–2**). Best results are in patients younger than 20 years of age with a related donor.

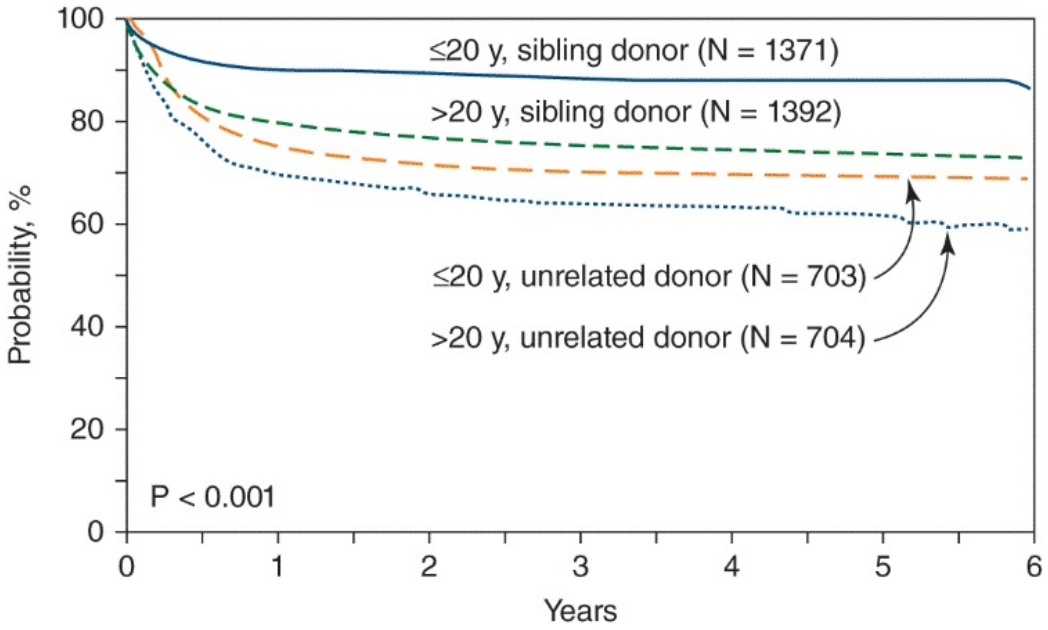


FIGURE 3–2 Probability of survival after hematopoietic stem cell transplantation for severe aplastic anemia by donor type and age, 1998–2008. Patients receiving marrow from a matched sibling had better outcomes if they were 20 years of age or younger, compared to those older than 20 years of age. Patients receiving marrow from a matched sibling fared better than those who received marrow from a matched unrelated donor, at any age. Patients younger than 20 or older than 20 years of age did not have a significant difference in outcome if they received marrow from an unrelated matched donor. (Reproduced with permission from Pasquini MC, Wang Z. Current uses and outcomes of hematopoietic stem cell transplantation: CIBMTR Summary Slides, 2013.)

Immunosuppressive Therapy

- Is the most successful therapy in patients unsuitable for allogeneic hematopoietic stem cell transplantation (see **Table 3–7**)
- Antithymocyte globulin (ATG) or antilymphocyte globulin (ALG)
  - — ATG prepared in horses or rabbits from human thymocytes and ALG prepared in horses or rabbits from human thoracic duct lymphocytes
  - — Fifty percent response rate when used as single agent
  - — Dose: 15 to 40 mg/kg daily intravenously for 4 to 10 days
  - — Fever and chills common on first day of treatment
  - — Accelerated platelet destruction with thrombocytopenia frequent during infusion
  - — Serum sickness possible with fever, rash, and arthralgias 7 to 10 days after beginning treatment
  - — Moderate dose of methylprednisolone usually used to decrease allergic reactions
- Cyclosporine
  - — Treatment in patients if refractory to ATG
  - — Dose: 3 to 7 mg/kg daily orally for at least 4 to 6 months
  - — Dose adjusted to maintain appropriate blood levels (trough blood levels of 300–500 ng/mL)
  - — Renal impairment: common side effect
  - — Response in 25% of patients overall
- Combination therapy: ATG and cyclosporine, which yield an significantly improved response rate over either agent alone
- High-dose glucocorticoids
  - — For example, 5 to 10 mg/kg methylprednisolone for 3 to 14 days

— Very severe side effects: glycosuria, gastric distress, insomnia, psychosis, infection, aseptic necrosis of the femoral head
— Little evidence for efficacy of glucocorticoids used alone
— Usually used in lower doses (2 mg/kg and then taper) as adjunct to ATG

- High-dose cyclophosphamide (eg, 45 mg/kg per day for 4 doses)
- Androgen therapy
  — Danazol, 5 mg/kg per day for 6 months, as primary therapy not efficacious in severe or moderate aplastic anemia
  — Androgen therapy combined with ALG and cyclosporine being assessed
  — Can induce severe masculinization and liver damage
- G-CSF as primary therapy not efficacious
  — Transient improvement in neutrophil counts observed with GM-CSF or G-CSF treatment in some patients but not sustained
  — G-CSF used in combined therapy with ATG and cyclosporine: no improved remission or survival rates in most studies
- Interleukin (IL)-3 or IL-1 as primary treatment not effective
- Results of combined immunosuppressive (ATG and cyclosporine therapy)
  — Marked hematologic improvement in 60% to 80% of patients
  — Possible long-term problems after immunosuppressive therapy, such as continued moderate anemia and thrombocytopenia, recurrent aplasia, PNH, acute myelogenous leukemia, or myelodysplastic syndrome
- Eltrombopag. This thrombopoietin receptor agonist has been used in patients who were not successfully treated with combined immunotherapy. A significant portion (~45%) had moderate to marked improvement in one or more blood cell counts or loss of red cell and platelet transfusion requirements. A few returned to normal blood cell counts. In some so treated, these effects have persisted for more than a year of observation. The initial studies started with a dose of 50 mg, increasing to 150 mg/day over a 12-week period. Longer treatment periods and higher doses may induce remissions in some patients. These variations in treatment are currently under study.

TABLE 3–7 **RESPONSE TO IMMUNOTHERAPY IN PATIENTS WITH SEVERE APLASTIC ANEMIA**

Year of Publication	Principal Drugs Used	No. Pts (Age range [Years])	Significant Response No. (%)	Survival at 5/10 Years (%)	Relapse at 5 Years (Cum%)	Comments
2011	ATG + CYA ATG + CYA + G-CSF	95 (7–80) 97 (2–81)	63 (66) 71 (73)	76*/NR 78*/NR	33* 32*	Fewer early infections with G-CSF; no difference in response or survival
2008	ATG + CYA	77 (< 18)	57 (74)	83/80	25	8.5% evolved to clonal myeloid disease
2007	ATG + CYA	44 (NR)	31 (70)	NR/88	NR	All cases were associated with hepatitis
2007	ATG + CYA	47 (19–75)	31 (66)	80/NR	45	No late clonal diseases at 5 years
2007	ATG + CYA + G-CSF	48 (19–74)	37 (77)	90/NR	15	No late clonal diseases at 5 years
2006	ATG + CYA	47 (8–71)	37 (79)	80/75	NR	No late clonal diseases at 10 years

2006	ATG + CYA + G-CSF + rhuEPO	30 (5–68)	22 (73)	80/75	NR	One patient developed clonal myeloid disease

ATG, antithymocyte globulin; Cum%, cumulative percent; CYA, cyclosporine; G-CSF, granulocyte colony-stimulating factor; No. Pts, number of patients; NR, not reported; rhuEPO, recombinant human erythropoietin.

*At 6 years post-treatment.

Source: *Williams Hematology*, 9th ed, Chap. 35, Table 35–7.

Supportive Care

- Immediate human leukocyte antigen (HLA) typing of patient and siblings as possible stem cell donors
- Minimal or no transfusions in potential transplant recipients, if possible
- If transfusions are needed, no use of family donors in a potential transplant recipient
- Transfusion of platelets based on assessment of risk of bleeding and not solely on platelet count
- Use of leukocyte-depleted, ABO blood group-compatible single-donor platelets, if possible, to minimize HLA sensitization, subsequent refractoriness, and other problems
- Aminocaproic acid, 4 to 12 g/d. This may decrease thrombocytopenic bleeding
- Transfusion of packed red blood cells (irradiated, leukocyte-depleted) when hemoglobin level is less than 8 g/dL. Use a higher threshold if comorbid conditions require
- Cytomegalovirus (CMV) serology for prospective transplant recipients. Transfuse only CMV-negative blood products until these results are known. If the patient is CMV-positive, these precautions can be discontinued. Use of leukocyte depletion filters also decreases risk of CMV acquisition
- Neutropenic precautions for hospitalized patients with absolute neutrophil counts of less than 500/mL
- Prompt institution of broad-spectrum intravenous antibiotics for fever after appropriate cultures have been obtained

Clinical Course

- Median survival of untreated severe aplastic anemia is 3 to 6 months (20% survive longer than 1 year). Allogeneic hematopoietic transplantation can cure a very large proportion of patients depending on their age at transplantation and the immunologic similarity of the donor (see **Figure 3–3**). Combined immunotherapy with ATG and cyclosporine can result in 10-year survival rate of 70% to 80%.

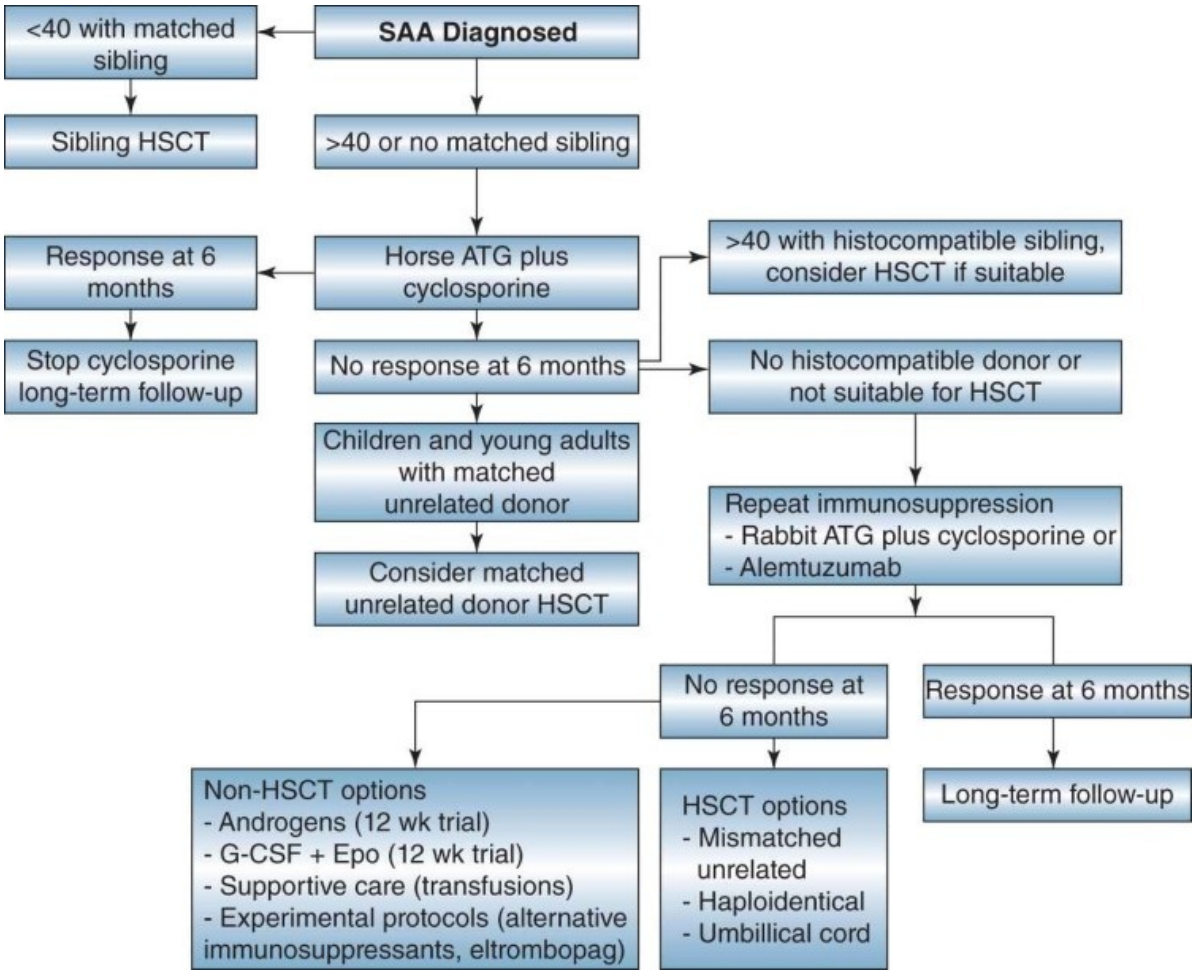
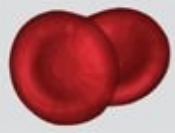


FIGURE 3–3 Flow chart with general guidelines for treatment of severe aplastic anemia (SAA) as of 2012.* Response to horse antithymocyte globulin (ATG) plus cyclosporine is followed for 6 months before deciding the patient has not responded adequately unless the patient is doing poorly and the neutrophil count remains less than 200×10^9/L. In that case, one can proceed to next suitable option. In general, transplantation options are reassessed at 6 months after immunotherapy and are dependent on donor availability and quality of match, patient age, comorbid conditions that would increase transplantation risk, and the severity of the depression in neutrophil count. In younger patients, a matched unrelated donor may be appropriate. In older patients, retreating with immunotherapy would be favored unless the neutrophil count persists in the very severe risk category. After two unsuccessful attempts at immunotherapy, therapy is individualized and a high-risk transplantation procedure (slight mismatched-related, haploidentical, umbilical cord blood) may be considered, using the relevant variables (eg, age, comorbidities, performance status, neutrophil count). The age of 40 years is an approximate guideline for considering an initial allogeneic hematopoietic stem cell transplant (HSCT) and may be modified upward somewhat (eg, 41–50 years) based on the clinical status and other features of the patient. *Based on observations reported in 2014, it would be appropriate to consider eltrombopag therapy, 3 to 6 months after the failure of combined immunotherapy with ATG and cyclosporine, before going to next options outlined in algorithm in this figure. At this writing, the Food and Drug Administration has not approved eltrombopag for use in patients with aplastic anemia, but such approval is anticipated. (Reproduced with permission from Scheinberg P, Young NS: How I treat acquired aplastic anemia, *Blood* 2012 Aug 9;120(6):1185-1196.)



For a more detailed discussion, see George B. Segel and Marshall A. Lichtman: Aplastic Anemia: Acquired and Inherited, Chap. 35 in *Williams Hematology*, 9th ed.

CHAPTER 4
Pure Red Cell Aplasia

DEFINITION

- *Pure red cell aplasia* describes isolated anemia secondary to failure of erythropoiesis. Cardinal findings are a low hemoglobin level combined with reticulocytopenia and absent or extremely infrequent marrow erythroid precursors.

CLASSIFICATION

- See **Table 4–1**.

TABLE 4–1 CLASSIFICATION OF PURE RED CELL APLASIA

Fetal red cell aplasia (nonimmune hydrops fetalis)
Parvovirus B19 in utero
Inherited (Diamond-Blackfan anemia): *RPS19* and other *RPS* mutations
Acquired
Transient pure red cell aplasia
Acute B19 parvovirus infection in hemolytic disease (transient aplastic crisis; ~100% of cases)
Transient erythroblastopenia of childhood
Chronic pure red cell aplasia
Idiopathic
Large granular lymphocytic leukemia
Chronic lymphocytic leukemia
Clonal myeloid diseases (especially 5q-syndrome)
Persistent B19 parvovirus infection in immunodeficient host (~15% of cases)
Thymoma
Collagen vascular diseases
Post–stem cell transplantation
Anti-ABO antibodies
Drug induced
Antierythropoietin antibodies
Pregnancy

Source: *Williams Hematology*, 9th ed, Chap. 36, Table 36–1.

INHERITED PURE RED CELL APLASIA (DIAMOND-BLACKFAN ANEMIA)

- This form of pure red cell aplasia, which occurs early in childhood, is also known as either Diamond-Blackfan or Blackfan-Diamond anemia.
- It has an estimated annual incidence of five cases per 1 million live births.
- Inheritance is usually autosomal dominant or occasionally autosomal recessive if a familial

pattern. Sporadic cases are most frequent.
- In this disease of abnormal ribosomal biogenesis, mutations involve the *RPS19* gene in about 25% of cases; several other genes that regulate ribosome assembly have been implicated.
- Pathophysiology is unclear.

Clinical Features

- Presenting symptoms include pallor, listlessness, poor appetite, and failure to thrive.
- One third of patients are diagnosed at birth or in the early neonatal period, but the disease may appear at any time into adulthood.
- Physical abnormalities occur in one third of patients (eg, craniofacial dysmorphism, short stature, abnormalities of the thumb, web neck, and urogenital and cardiac abnormalities).
- Disease may progress to severe anemia, with cardiac failure, dyspnea, and hepatosplenomegaly.

Laboratory Features

- Absolute severe reticulocytopenia occurs in all cases.
- Normocytic, occasionally macrocytic, normochromic anemia is found.
- Leukocyte count is normal or slightly decreased. Neutropenia may develop over several years.
- Platelet count is normal or mildly increased.
- Marrow is cellular but with marked erythroid hypoplasia. The few erythroid cells present may have megaloblastic changes. Other marrow cells are normal.
- Serum iron levels are elevated, and transferrin saturation is increased.
- Erythropoietin levels are elevated.
- Erythrocyte adenosine deaminase activity is elevated in 75% of patients.

Differential Diagnosis

- Characteristic triad includes anemia, reticulocytopenia, and paucity/absence of marrow erythroid precursors. Findings are supplemented by increased erythrocyte adenosine deaminase activity and *RPS19* gene mutations.
- Fanconi anemia can be excluded by cytogenetic and gene mutation analyses.
- Transient erythroblastopenia of childhood is established by spontaneous recovery.

Therapy, Course, and Prognosis

- Transfusions relieve symptoms of anemia but lead to iron overload. Iron chelation therapy should be initiated promptly (Chap. 9). Transfusions should be leukocyte depleted to avoid alloimmunization (Chap. 91).
- Glucocorticoid therapy may be beneficial, although its mechanism of action is unclear. Response is not predictable.
- Glucocorticoid therapy should be initiated with prednisone at a daily dose of 2 mg/kg per day, orally, in three or four divided doses. A reticulocyte response is usually seen within 1 to 4 weeks. Once the hemoglobin level reaches 9 to 10 g/dL (90–100 g/L), the initial dose is reduced very slowly to a single daily dose, then to an alternate-day schedule. The goal is to get to low (1–2 mg/day), alternate-day therapy. Continuous therapy is typically required,

because withdrawal of glucocorticoids is often, but not always, accompanied by relapse.

- Severe side effects from glucocorticoid therapy frequently develop (eg, Cushing syndrome). Long-term transfusions with iron chelation may be preferable to long-term higher dose glucocorticoids and resultant side effects.
- Allogeneic hematopoietic cell transplantation from a histocompatible sibling, when successful, is curative. Allogeneic transplantation from unrelated donor sources, including umbilical cord blood, has been less successful. However, transplantation has usually been utilized late in the disease course due to its accompanying risks of morbidity and mortality.
- High-dose methylprednisolone, immunosuppressive agents, and interleukin-3 have been reported to ameliorate the disease but are not standard therapies.
- Most deaths are a result of therapeutic complications from chronic iron overload, hypercorticism, or hematopoietic cell transplantation.
- Patients have developed acute myelogenous leukemia at a higher rate than expected.

TRANSIENT APLASTIC CRISIS AND TRANSIENT ERYTHROBLASTOPENIA OF CHILDHOOD

- This condition is clinically identical to pure red cell aplasia except for spontaneous resolution of symptoms and laboratory findings.

Etiology

- Most patients with aplastic crises are infected with B19 parvovirus (**Figure 4–1**), typically in the context of an underlying hemolytic disease such as hereditary spherocytosis or sickle cell disease (transient aplastic crisis).
- This occurs in normal children after an infection by an unknown childhood virus (transient erythroblastopenia of childhood).
- Drugs implicated in chronic pure red cell aplasia may also induce transient aplastic crises.

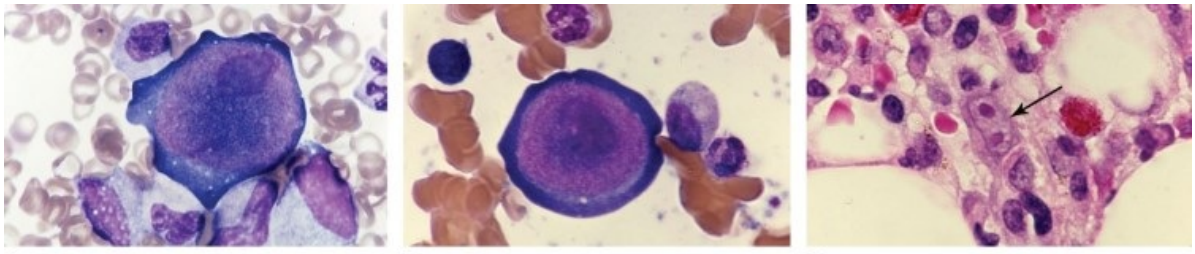


FIGURE 4–1 **A** and **B.** Giant early erythroblast precursors in the marrow aspirate of a patient with chronic pure red cell aplasia secondary to persistent B19 parvovirus infection. Note the nuclear inclusions (darker nuclear shading) representing parvovirus infection. **C.** Marrow biopsy section. The arrows point to binucleate erythroid precursor cell with nuclear inclusions representing parvovirus infection. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

Clinical Features

- Transient aplastic crisis in the context of an underlying hemolytic disease results in more evident pallor, fatigue on exertion, and lassitude. Gastrointestinal complaints or headache may be associated symptoms. Physical examination findings may include tachycardia and a flow murmur.

- Transient erythroblastopenia of childhood presents as an acute anemia in a previously well child. Rare complications include seizures and transient neurologic abnormalities.

Laboratory Features

- In both syndromes, anemia is the hallmark, and hemoglobin levels may be markedly depressed. Red cell indices are normal.
- Reticulocytes are absent; marrow erythroid precursor cells are absent or markedly decreased.
- Granulocyte and platelet counts are normal or elevated.
- Reticulocytosis is the first sign of recovery, and some nucleated red cells may appear in the blood, transiently.

Differential Diagnosis

- Absent reticulocytes distinguish increasing anemia in a patient with hemolytic disease as transient aplastic crisis.
- Transient erythroblastopenia of childhood is differentiated from inherited red cell aplasia by older age, no family history, lack of physical anomalies, and spontaneous resolution.
- A record of current medications (more important in adults) may provide clues to a drug-induced crisis rather than idiopathic disease.

Therapy, Course, and Prognosis

- Severe anemia may require red blood cell transfusion.
- Recovery from B19 parvovirus infection occurs spontaneously in days or weeks as neutralizing antibodies are made.
- Transient erythroblastopenia of childhood typically resolves after a few weeks. Overtreatment of a self-limited illness should be avoided.
- For drug-associated aplasia, discontinuation of the offending drug results in clinical improvement.

ACQUIRED CHRONIC PURE RED CELL APLASIA

- An uncommon disorder, typically of older adults, this type of aplasia is characterized by anemia, severe reticulocytopenia, and absent marrow erythroid precursor cells.

Etiology

- Immune-mediated erythropoietic failure results from antibody inhibition or T-cell–mediated suppression of erythropoiesis. The latter mechanism is more common. Red cell aplasia is associated with autoimmune diseases, thymoma, lymphoproliferative processes, and pregnancy.
- In the absence of an effective antibody response, B19 parvovirus infection may persist and cause pure red cell aplasia.
- Rarely, red cell aplasia, as a result of an intrinsic cellular defect, can be the first or the major manifestation of myelodysplasia.

- Idiosyncratic drug reactions are an uncommon cause.

Clinical Features

- Pallor, lassitude, and other signs and symptoms of anemia are usual.
- Concomitant diseases (chronic lymphocytic leukemia, lymphoma, autoimmune diseases) may be present.

Laboratory Features

- Blood shows normochromic, normocytic or macrocytic anemia with severe reticulocytopenia and a normal leukocyte and platelet count.
- The marrow is normocellular, with normal granulocytes and megakaryocytes, but with severe erythroid hypoplasia or aplasia.
- The serum iron level is elevated, and the iron-binding capacity almost fully saturated.
- Thymic enlargement, if present, may be detected as an anterior mediastinal mass on routine chest films. If not, computed tomography may be required to determine if a thymoma is present (uncommon).
- Persistent parvovirus infection may be diagnosed by the presence of parvovirus DNA in the blood.

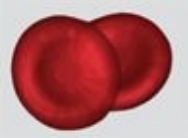
Differential Diagnosis

- Distinction between inherited and acquired red cell aplasia may be impossible in the younger patient.
- Dysplastic changes in neutrophils, their precursors and in megakaryocytes, with accompanying cytopenias, can be a rare accompanying feature of a myelodysplastic syndrome.
- B19 parvovirus infection should always be suspected in any immunocompromised individual, including following hematopoietic cell transplantation.

Therapy, Course, and Prognosis

- Red cell transfusions and iron chelation are basic to management. Two units of red cells every 2 weeks may keep nadir hemoglobin above 7 g/dL (70 g/L). A higher nadir may be required if comorbidities exist.
- Immunosuppressive agents are used to treat disease of suspected immune origin. Response is likely, but sequential treatment with a variety of agents is often required. About half of patients respond to oral prednisone 1 to 2 mg/kg/day. Some advocate cyclosporine as first-line therapy because higher response rates have been reported. Monoclonal antibodies, including daclizumab, rituximab, and alemtuzumab may be efficacious. Some patients with resistant disease respond to fludarabine and cladribine. Plasmapheresis has been reported to be of long-lasting benefit in a few patients.
- In the case of a thymoma, thymectomy should be considered to prevent malignant metastasis but does not necessarily improve marrow function. Cyclosporine is the most effective drug to treat pure red cell aplasia associated with thymoma.
- Intravenous gamma globulin at a dose of 0.4 g/kg/day for 5 to 10 days is effective in treating persistent B19 parvovirus infection. Retreatment or maintenance therapy may be required for

some patients.
- Normal life expectancy can be achieved even in refractory cases.



For a more detailed discussion, see Neal S. Young: Pure Red Cell Aplasia, Chap. 36 in *Williams Hematology*, 9th ed.

CHAPTER 5
Anemia of Chronic Disease

ANEMIA OF CHRONIC RENAL DISEASE

Etiology and Pathogenesis

- Reduced renal production of erythropoietin (EPO) and inflammation and hepcidin-induced iron restriction are the most significant factors in the development of anemia in renal insufficiency.
- A modest reduction in red cell life span occurs in uremia, probably as a result of metabolic impairment of red cells.
- Iron deficiency occurs from blood loss in dialysis tubing, laboratory testing, or external bleeding, sometimes as a result of uremia-induced platelet dysfunction. Furthermore, increased hepcidin blocks iron absorption in the gut and iron release from macrophage stores (in part ameliorated by therapy with EPO or other erythropoiesis-stimulating agents [ESAs]).
- The plasma volume varies widely in renal failure, with consequent variations in the hemoglobin concentration.

Clinical and Laboratory Features

- The anemia is normocytic and normochromic with a reduced blood concentration of reticulocytes relative to the degree of anemia. Gastrointestinal and gynecologic bleeding occurs in one third to one half of all patients with chronic renal failure.
- Acanthocytes or schistocytes may be seen on the blood film.
- Total and differential leukocyte count and platelet count are usually normal.
- Platelet function is abnormal, in relationship to the degree of uremia.
- Cellularity and blood cell maturation sequences in the marrow are normal. Despite the anemia, there is no compensatory erythroid hyperplasia.

Therapy, Course, and Prognosis

- Replacement therapy with EPO or other ESA corrects the anemia in nearly all patients. Amelioration of the anemia improves the quality of life for uremic patients.
- EPO and iron are usually given intravenously in dialysis patients. A target hemoglobin level of 10 to 11 g/dL is recommended.
- Adequate iron and folate supply should be maintained to achieve an optimal response with ESA therapy. If transferrin iron saturation is less than 30% and ferritin is less than 300 ng/mL, intravenous iron therapy usually increases hemoglobin levels or decreases ESA doses required, whether or not patients are on hemodialysis.
- Long-acting ESA preparations (eg, darbepoetin) given subcutaneously may be more convenient and, perhaps, safer for patients not undergoing dialysis because plasma ESA levels are lower

but more sustained.

- Complications of EPO therapy include hypertension, seizures, increased cardiovascular morbidity and mortality and thrombosis of shunts; hemoglobin levels of greater than 11 g/dL should be avoided. Blood pressure should be carefully monitored throughout the treatment.
- A small number of patients do not respond to EPO or require higher doses, most often because of iron deficiency, infection, or inflammation. Common causes of EPO hyporesponsiveness are listed in **Table 5–1**.
- Chronic hemodialysis may improve the platelet dysfunction.

TABLE 5–1 COMMON CAUSES OF ERYTHROPOIETIN HYPORESPONSIVENESS

Infection, inflammation
Cancer, administration of chemotherapy or radiotherapy
Hyperparathyroidism
Iron deficiency
Folate deficiency
Sickle cell anemia
Thalassemia
Other hemolytic anemia
Myelodysplastic syndrome

ANEMIA OF INFLAMMATION

Definition

- Anemia of inflammation (AI) is associated with (1) infection or (2) inflammatory or neoplastic disease.
- AI is also referred to as anemia of chronic disease.
- In nonhospitalized patients, 1 to 2 months of sustained disease is required for anemia to develop.
- During critical illness, anemia similar to AI can develop much more rapidly, accelerated by frequent diagnostic phlebotomy, other occult blood loss, or suppression of erythropoiesis by high concentrations of circulating inflammatory cytokines and shortening of erythrocyte survival.
- The hemoglobin level is usually between 7 and 11 g/dL, and in the higher range (10–11 g/dL), it may be asymptomatic.
- The characteristic laboratory features of AI are listed in **Table 5–2**.

TABLE 5–2 LABORATORY STUDIES OF IRON METABOLISM IN IRON-DEFICIENCY ANEMIA (IDA) AND ANEMIA OF INFLAMMATION (AI)

	IDA (n = 48)	AI (n = 58)	Combination (n = 17)
Hemoglobin, g/L	93 ± 16 (96)	102 ± 12 (103)	88 ± 20 (90)
MCV, fL	75 ± 9 (75)	90 ± 7 (91)	78 ± 9 (79)
Iron, µmol/L (n = 10–40)	8 ± 11 (4)	10 ± 6 (9)	6 ± 3 (6)
Transferrin, g/L (n = 2.1–3.4 m, 2.0–3.1 f)	3.3 ± 0.4 (3.3)	1.9 ± 0.5 (1.8)	2.6 ± 0.6 (2.4)
Transferrin saturation, %	12 ± 17 (5.7)	23 ± 13 (21)	12 ± 7 (8)
Ferritin, µg/L (n = 15–306 m, 5–103 f)	21 ± 55 (11)	342 ± 385 (195)	87 ± 167 (23)

TfR, mg/L (n = 0.85–3.05)	6.2 ± 3.5 (5.0)	1.8 ± 0.6 (1.8)	5.1 ± 2.0 (4.7)
TfR/log ferritin	6.8 ± 6.5 (5.4)	0.8 ± 0.3 (0.8)	3.8 ± 1.9 (3.2)

f, female; m, male; n, normal; TfR, transferrin receptor.

Diagnosis was defined by marrow iron stain and appropriate coexisting disease. Patients with a combination of no stainable marrow iron and either coexisting disease or elevated CRP were classified as "COMBINATION." Normal ranges for this laboratory for males (m) and females (f) are indicated. Measurements are presented as mean ± SD (median).

Reproduced with permission from Punnonen K, Irjala K, Rajamäki A: Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency, *Blood* 1997 Feb 1;89(3):1052-1057.

Pathogenesis

- Inflammation leads to interleukin (IL)-6 production, which induces hepatocyte hepcidin production, which in turn blocks intestinal iron absorption and iron release from macrophages and hepatocytes. Hepcidin binds to ferroportin, the primary cell surface iron exporter, and causes its degradation.
- Impaired intestinal iron uptake and impaired release of iron from macrophages lead to a low level of serum iron and consequent low saturation of transferrin.
- Enhanced activity of macrophages increases rate of erythrocyte destruction.
- Production of EPO is decreased in response to anemia, and the ability of erythroid precursors to respond to EPO is impaired. Both of these are also related to inflammatory cytokine production (IL-1, tumor necrosis factor, interferons).

Clinical and Laboratory Features

- Symptoms of anemia, if mild, are usually overshadowed by symptoms of the primary disease.
- Common conditions leading to AI are shown in **Table 5–3**.
- There is a low reticulocyte index for the degree of anemia.
- Diagnosis, especially differentiation from iron-deficiency anemia (IDA), depends on laboratory findings (see **Table 5–2**).
  - — Initially there is normochromic, normocytic anemia; hypochromic, microcytic features develop as anemia progresses.
  - — Low serum iron level and somewhat decreased serum transferrin concentration occur, with decreased percent transferrin saturation.
  - — Level of serum ferritin, an acute phase protein, is elevated.
  - — Marrow contains increased storage iron, but the percentage of normal sideroblasts is decreased.

TABLE 5–3 COMMON CONDITIONS ASSOCIATED WITH ANEMIA OF INFLAMMATION

Category	Diseases Associated with Anemia of Inflammation
Infection	AIDS/HIV, tuberculosis, malaria (contributory), osteomyelitis, chronic abscesses, sepsis
Inflammation	Rheumatoid arthritis, other rheumatologic disorders, inflammatory bowel diseases, systemic inflammatory response syndrome
Malignancy	Carcinomas, myeloma, lymphomas
Cytokine dysregulation	Anemia of aging

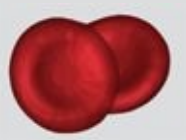
Source: *Williams Hematology,* 9th ed, Chap. 37, Table 37–1.

Differential Diagnosis

- Drug-induced marrow suppression or drug-induced hemolysis
- Iron-deficiency anemia characterized by low serum iron, increased transferrin, decreased transferrin saturation, absent storage iron, and markedly decreased serum ferritin
- Anemia of chronic renal failure
- Myelophthisic anemia caused by carcinoma or lymphoma replacing marrow hematopoietic tissue

Therapy

- No treatment may be necessary, other than for the underlying disease.
- Packed red cell transfusions may be given, if the anemia is symptomatic.
- Although rarely indicated, EPO therapy may be effective especially when given in combination with intravenous iron, as in anemia of chronic renal disease.
  — Hypertension and a risk of thrombotic complications occur with use of EPO preparations.



For more detailed discussion, see Tomas Ganz: Chap. 37 in *Williams Hematology*, 9th ed.

CHAPTER 6

Anemia of Endocrine Disorders

- Anemia due to endocrine disease is generally mild to moderate; however, a decreased plasma volume in some of these disorders may mask the severity of the decrease in red cell mass.
- The pathophysiologic basis of the anemia seen in endocrine disorders is often multifactorial.

THYROID DYSFUNCTION

- Anemia in hypothyroidism may be normocytic, macrocytic, or microcytic; coexisting deficiencies of iron, B_{12}, and folate may explain some of this heterogeneity.
- Iron deficiency often occurs in hypothyroidism as a result of increased predisposition to menorrhagia, an associated achlorhydria, or because a deficit of thyroid hormone may decrease iron absorption.
- In patients with coexisting iron-deficiency anemia and subclinical hypothyroidism, the anemia often does not adequately respond to oral iron therapy.
- The mechanism underlying the association of hypothyroidism and pernicious anemia is unknown.
- The mean corpuscular volume cannot be used to differentiate hypothyroid patients with low vitamin B_{12} levels from those with uncomplicated hypothyroidism.
- Anemia is also a direct consequence of thyroid hormone deficiency; thyroid hormones have been shown to potentiate the effect of erythropoietin on erythroid colony formation.
- Patients with hyperthyroidism have increased red cell mass, but the hematocrit and hemoglobin concentration are usually not elevated because the plasma volume is also increased.
- Autoimmune hemolytic anemia and pancytopenia responsive to treatment of hyperthyroidism have also been reported.

ADRENAL GLAND DISORDERS

- The red cell mass is decreased in primary adrenal insufficiency (Addison disease), but it may not be reflected in the hematocrit or hemoglobin measurements because of a concomitant reduction in plasma volume.
- The pathophysiologic basis of the anemia and any influence of adrenal cortical hormones on erythropoiesis are not well defined.
- Some patients with Addison disease develop a transient fall in hematocrit and hemoglobin concentration after initiation of hormone replacement therapy (presumably secondary to an increased plasma volume).

- Pernicious anemia occurs in patients with autoimmune adrenal insufficiency, but is seen primarily in patients with type I polyglandular autoimmune syndrome, whose other manifestations include mucocutaneous candidiasis and hypoparathyroidism.
- Polycythemia has been reported in Cushing syndrome, primary aldosteronism, Bartter syndrome, and congenital adrenal hyperplasia secondary to 21-hydroxylase deficiency.
- Conversely, some males with Cushing syndrome are anemic; this finding is correlated with a low testosterone level.
- Some individuals with congenital polycythemia develop recurrent pheochromocytomas, paragangliomas, and somatostatinomas that are heterozygous for a gain-of-function mutation of hypoxia-inducible factor 2α. However, the association of these tumors with polycythemia is unknown.

GONADAL HORMONES

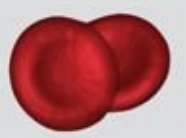
- Decrease in androgen production due to orchiectomy or medical androgen blockade causes anemia.
- Androgen therapy has been used for the treatment of various anemias, especially before the development of recombinant erythropoietin.
- The mechanism of androgen action appears to be complex, with evidence for stimulation of erythropoietin secretion and a direct effect on the marrow erythroid progenitors.
- Estrogens in large doses cause moderately severe anemia by a mechanism not clearly defined.

PITUITARY GLAND DISORDERS

- Hypopituitarism results in a moderately severe normochromic normocytic anemia, with an average hemoglobin of 10 g/dL.
- Anemia of hypopituitarism results from the absence of the anterior lobe hormones, which include adrenocorticotropic hormone, thyroid-stimulating hormone, follicle-stimulating hormone, luteinizing hormone, growth hormone, and prolactin. The resulting deficiencies of thyroid hormones, adrenal hormones, and androgens are likely the major contributors to anemia.
- Red cell survival is normal in hypopituitarism. However, the marrow is hypoplastic, and leukopenia or pancytopenia can also occur.
- Replacement therapy with a combination of thyroid, adrenal, and gonadal hormones usually corrects the anemia and other cytopenias in hypopituitarism.
- Erythropoietin therapy may be effective in postoperative hypopituitarism refractory to hormone replacement therapy.
- Children with isolated growth hormone deficiency become anemic, which is improved with growth hormone replacement therapy.
- Macroprolactinomas have been associated with anemia, likely due to a concomitant decrease in testosterone levels.
- Pituitary adenomas that secrete gonadotropins are rare but have been associated with erythrocytosis, likely due to testosterone excess.

HYPERPARATHYROIDISM

- A normochromic and normocytic anemia not attributable to other causes is present in 3% to 5% of patients with primary hyperparathyroidism.
- The cause of the anemia is unknown, but marrow fibrosis has been described in a few patients.
- Secondary hyperparathyroidism in patients with renal failure may contribute to refractoriness to erythropoietin therapy.



For a more detailed discussion, see Xylina T. Gregg: Anemia of Endocrine Disorders, Chap. 38 in *Williams Hematology*, 9th ed.

CHAPTER 7
Congenital Dyserythropoietic Anemias

- Congenital dyserythropoietic anemias (CDAs) are a heterogeneous group of disorders characterized by anemia, ineffective erythropoiesis with specific morphological alterations of erythroid precursors in the marrow, and iron overload.
- Although rare, uncovering the molecular basis of CDA has helped unravel novel aspects of the cell biology of erythropoiesis.
- Three classical types of CDA have been distinguished. However, some patients with phenotype of CDA do not fit into any of these categories.

CDA TYPE I

Clinical and Laboratory Features

- The condition presents in infancy or adolescence.
- Autosomal recessive inheritance is caused by mutations in the *CDAN1* gene, encoding codanin-1, a cell cycle–regulated protein involved in the histone assembly; homozygosity is often associated with consanguinity. In a number of patients, only one or no *CDAN1* mutated allele is identified; other causative genes, such as *C15ORF41*, are suspected.
- Moderately severe macrocytic anemia (approximately 9.0 g/dL) occurs.
- Hepatomegaly and cholelithiasis are common.
- Splenomegaly increases with age.
- Specific morphologic findings of CDA type I are summarized in **Table 7–1** and exemplified in **Figure 7–1**.
- Dysmorphic skeletal features may be present, typically affecting hands and feet. Less common are small stature, almond-shaped blue eyes, hypertelorism, and micrognathia.

TABLE 7–1 CARDINAL FEATURES OF TYPES I, II, AND III CONGENITAL DYSERYTHROPOIETIC ANEMIAS (CDAS)

CDA Type	Light Microscopy	Electron Microscopy	Serology	Inheritance
I	Most erythroid cells abnormal: double nuclei, internuclear chromatin bridges (Fig. 7–1)	Widened nuclear pores, spongy appearance of the heterochromatin, invasion by the cytoplasm containing various organelles	No serologic abnormalities	Autosomal recessive
II	Mature stage erythroblasts with two or more nuclei, lobulated nuclei, karyorrhexis, pseudo-Gaucher cells (Fig. 7–1)	Endoplasmic reticulum cisternae lining the inner surface of the red cell plasma membrane	Cells containing the HEMPAS antigen are lysed by 30% of acidified normal sera; strong reactivity with anti-“i” autoantibodies	Autosomal recessive

III	Giant erythroblasts, up to 50 μm in diameter, with up to 12 nuclei, basophilic stippling	Clefts and blebs within nuclear areas, some iron-filled mitochondria, autophagic vacuoles and myelin figures in the cytoplasm	No clearly defined abnormalities	Autosomal dominant (not all cases)

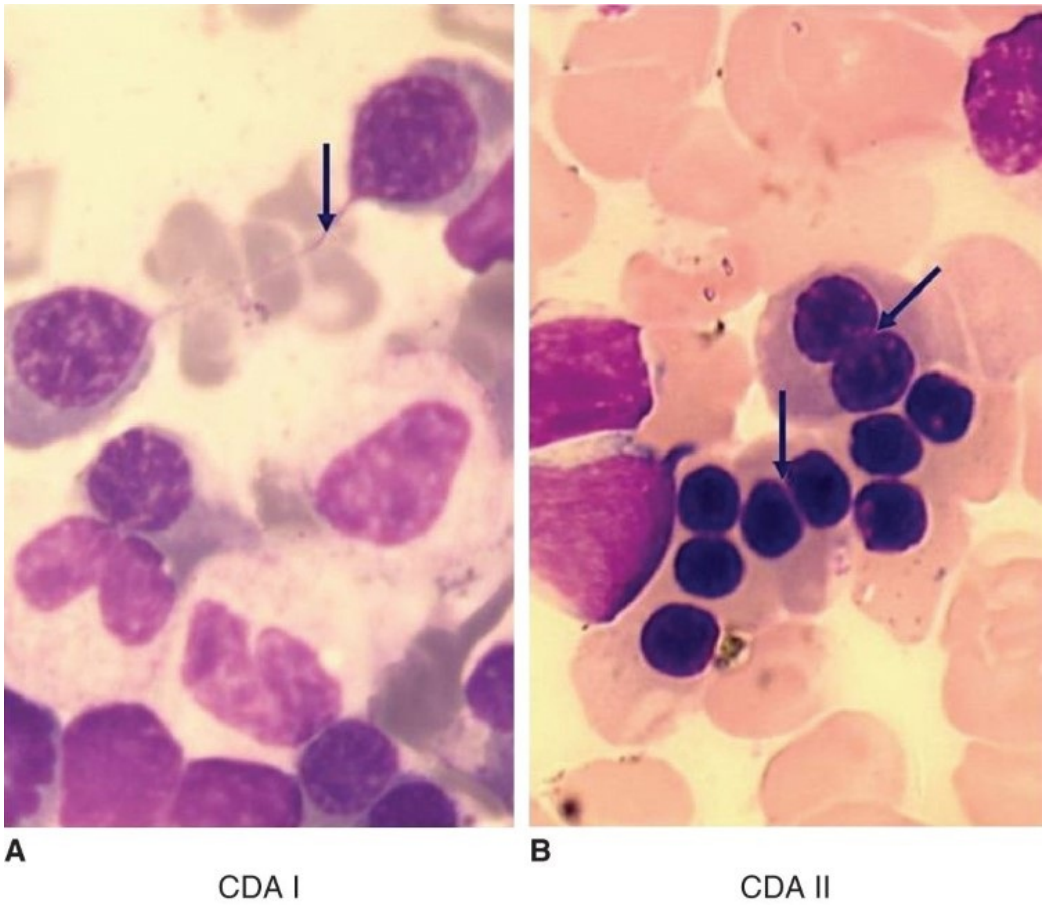


FIGURE 7–1 Light microscopy of marrow. **A.** Congenital dyserythropoietic anemia type I. The internuclear unusually long chromatin bridge is marked by an arrow. **B.** Congenital dyserythropoietic anemia type II. The two arrows point to binucleate erythroblasts, characteristic of this type. (Used with permission from Dr. Odile Fenneteau.)

Differential Diagnosis

- The condition may be confused with the thalassemias (see Chap. 15).
- Megaloblastoid marrow morphology may suggest folic acid or vitamin B_{12} deficiency (see Chap. 8).

Treatment

- Severe forms may present with hydrops fetalis. In severe cases, intrauterine red cell transfusions have been used (see Chap. 25).
- Red cell transfusions should be used judiciously because of the risk of iron overload.
- In patients with moderate anemia, small volume, regular phlebotomies, or chelating agents may be beneficial for iron overload (see Chap. 9).
- Some patients with CDA I have responded to α-interferon with improved anemia and decreased iron overload.

CDA TYPE II (HEMPAS)

- Type II CDA is also known by its acronym HEMPAS for *H*ereditary *E*rythroblastic *M*ultinuclearity associated with a *P*ositive *A*cidified *S*erum test.

Clinical and Laboratory Features

- Autosomal recessive inheritance is due to mutations in *SEC23B* gene, which encodes a component of the coat protein complex II (COPII), responsible for the biogenesis of endoplasmic reticulum-derived vesicles—a component of the *cis*-Golgi.
- Anemia varies from mild to severe.
- Moderate-to-marked anisocytosis, poikilocytosis, anisochromia, and contracted spherocytes are present in peripheral blood. Gaucher-like cells and ring sideroblasts may be found in the marrow (**Table 7–1**). The more than 10% of mature bi- and multinucleated red cell precursors is a morphologic hallmark (**Figure 7–1**).
- Iron stores are increased and symptomatic iron overload may occur even in those not transfused.

Treatment

- Red cell transfusions may be necessary. Iron chelation should be instituted when the ferritin level exceeds 500 to 1000 µg/L.
- Partial benefit may be at times seen with splenectomy.
- Marrow transplantation has been used in few patients and should be considered early before iron overload develops (see Chap. 39).

CDA TYPE III

Clinical and Laboratory Features

- Autosomal dominant inheritance is due to mutations in *KIF23* gene, encoding mitotic kinesin-like protein 1, which is critical for cytokinesis.
- Most patients are asymptomatic with mild to moderate anemia, mild jaundice, and, commonly, cholelithiasis.
- Some macrocytes may be extremely large ("gigantocytes"), and poikilocytes are present. The marrow has marked erythroid hyperplasia, with large multinucleate erythroblasts with big lobulated nuclei, and giant multinucleate erythroblasts (**Table 7–1**).

Treatment

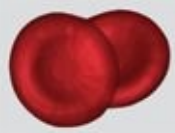
- Generally, none is needed. One symptomatic patient benefited from splenectomy.

ATYPICAL CONGENITAL DYSERYTHROPOIETIC ANEMIAS

Several rare types of congenital dyserythropoietic anemia do not conform to types I to III and are described in **Table 7–2**.

TABLE 7–2 CLASSIFICATION OF ATYPICAL CONGENITAL DYSERYTHROPOIETIC ANEMIAS (CDAS) NOT CONFORMING TO TYPES I, II, AND III

CDA Type/Syndromic CDA	Main Features
CDA Type IV (*KLF1* gene)	Autosomal dominant inheritance Normochromic, normocytic anemia of variable degree; hydrops fetalis, intrauterine transfusions, splenomegaly Elevated levels of hemoglobin F (> 30%) Hypercellular marrow, dysplastic changes in erythroblasts Electron microscopy: atypical cytoplasmic inclusions, enlarged nuclear pores, invagination of nuclear membrane
XLTDA (*GATA1* gene)	X-linked inheritance Macrothrombocytopenia with hypogranulated platelets, bleeding tendency Anemia of variable degree, ranging from hydrops fetalis and transfusion-dependency to dyserythropoiesis without anemia Bone marrow: megakaryocytes decreased in number, absence of platelet membrane demarcation, presence of cytoplasmic vacuoles
Majeed syndrome (*LPIN2* gene)	Autosomal recessive inheritance Chronic recurrent multifocal osteomyelitis, inflammatory dermatosis Microcytic anemia Bone marrow: dyserythropoiesis, erythroid hyperplasia, up to 25% bi- and tri-nucleated erythroblasts
CDA due to mutations in *COX4I2* gene	Exocrine pancreatic insufficiency and calvarial hyperostosis Blood smear: anisopoikilocytosis, basophilic stippling, and few normoblasts Bone marrow: erythroid hyperplasia, megaloblastic changes, bi- and multinucleated erythroblasts
CDA and mevalonate kinase deficiency (*MVK* gene)	Severe anemia; recurrent episodes of fever, rash, cervical adenitis, and abdominal pain Mildly dysmorphic facial features, including down-slanted palpebral fissures, hypertelorism, and frontal bossing Bone marrow: hypercellular with 6% to 8% of erythroid precursors exhibiting dyserythropoiesis, including forms of irregular nuclei, nuclear budding, binucleation, and karyorrhexis



For a more detailed discussion, see Achille Iolascon: The Congenital Dyserythropoietic Anemias, Chap. 39 in *Williams Hematology*, 9th ed.

CHAPTER 8
Folate, Cobalamin, and Megaloblastic Anemias

DEFINITION

- These disorders are caused by impaired synthesis of DNA.
- They most commonly result from folate or cobalamin (vitamin B_{12}) deficiency.
- Characteristics are megaloblastic cells, typically present in the erythroid series as large cells with immature-appearing nuclei but with increasing hemoglobinization of the cytoplasm—often referred to as *nuclear-cytoplasmic asynchrony*.
- Megaloblastic granulocytic cells have large size. Giant band neutrophils are a feature in the marrow with hypersegmented neutrophils in the marrow and blood. Megakaryocytes may be abnormally large with nuclear abnormalities.

ETIOLOGY AND PATHOGENESIS

- **Table 8–1** lists causes of megaloblastic anemia.
- By far the most common causes worldwide are folate deficiency and cobalamin deficiency.
- The underlying defect is impaired DNA synthesis because of failure of conversion of dUMP to dTMP.
- Intramedullary destruction of red cell precursors (*ineffective erythropoiesis*) is a major feature of megaloblastic anemia. Ineffective granulopoiesis and thrombopoiesis are also present and can result in neutropenia and thrombocytopenia. Ineffective hematopoiesis is characterized by marked hyperplasia of precursor cells (hypercellular marrow) with exaggerated apoptosis of late precursors, which results in blood cytopenias.
- Mild hemolysis also occurs; the red cell life span is reduced by about 40%.

TABLE 8–1 CAUSES OF MEGALOBLASTIC ANEMIAS

I. **Folate Deficiency**
  A. Decreased intake
    1. Poor nutrition
    2. Old age, poverty, alcoholism
    3. Hyperalimentation
    4. Hemodialysis
    5. Premature infants
    6. Spinal cord injury
    7. Children on synthetic diets
    8. Goat’s milk anemia
  B. Impaired absorption
    1. Nontropical sprue
    2. Tropical sprue
    3. Other disease of the small intestine

C. Increased requirements
1. Pregnancy
2. Increased cell turnover
3. Chronic hemolytic anemia
4. Exfoliative dermatitis

II. **Cobalamin Deficiency**
A. Impaired absorption
1. Gastric causes
a. Pernicious anemia
b. Gastrectomy
c. Zollinger-Ellison syndrome
2. Intestinal causes
a. Ileal resection or disease
b. Blind loop syndrome
c. Fish tapeworm
3. Pancreatic insufficiency
B. Decreased intake: vegans

III. **Acute Megaloblastic Anemia**
A. Nitrous oxide exposure
B. Severe illness with
1. Extensive transfusion
2. Dialysis
3. Total parenteral nutrition

IV. **Drugs**
A. Dihydrofolate reductase inhibitors
B. Antimetabolites
C. Inhibitors of deoxynucleotide synthesis
D. Anticonvulsants
E. Oral contraceptives
F. Others, such as long-term exposure to weak folate antagonists (e.g., trimethoprim or low-dose methotrexate)

V. **Inborn Errors**
A. Cobalamin deficiency
1. Imerslund-Gräsbeck disease
2. Congenital deficiency of intrinsic factor
3. Transcobalamin deficiency
B. Errors of cobalamin metabolism: "cobalamin mutant" syndromes with homocystinuria and/or methylmalonic acidemia
C. Errors of folate metabolism
1. Congenital folate malabsorption
2. Dihydrofolate reductase deficiency
3. N^5-methyl FH_4 homocysteine-methyltransferase deficiency
D. Other errors
1. Hereditary orotic aciduria
2. Lesch-Nyhan syndrome
3. Thiamine-responsive megaloblastic anemia

VI. **Unexplained**
A. Congenital dyserythropoietic anemia
B. Refractory megaloblastic anemia
C. Erythroleukemia

Source: *Williams Hematology,* 9th ed, Chap. 41, Table 41–4.

CLINICAL FEATURES

- Anemia develops gradually, and patients can adapt to very low hemoglobin levels. Eventually, as it progresses, the presenting symptoms are those of anemia with weakness, palpitation, fatigue, light-headedness, and shortness of breath.

- The condition may present initially with neurologic manifestations without anemia.
- Folic acid deficiency and cobalamin deficiency have indistinguishable blood and marrow changes (megaloblastosis), but the former deficiency is not associated with neuropathology and the latter characteristically is (see “Pernicious Anemia” below).

LABORATORY FEATURES

- All cell lines are affected.
- Erythrocytes show marked anisocytosis and poikilocytosis, with many oval macrocytes and, in severe cases, basophilic stippling, Howell-Jolly bodies, and Cabot rings. Erythrocytes with megaloblastic nuclei may be present in the blood (**Figure 8–1**).
- Absolute reticulocyte count is low.
- Anemia is usually macrocytic, with a mean cell volume (MCV)of 100 to 150 fL or more, but coexisting iron deficiency, thalassemia trait, or inflammation may prevent macrocytosis.
- Leukopenia and thrombocytopenia are frequently present.
- Hypersegmented neutrophils are an early sign of megaloblastosis. Typically, the nuclei of more than 5% of the cells have more than five lobes. Normal blood has less than 1% five-lobed neutrophils.
- Platelets are smaller than usual and vary more widely in size. Platelets are functionally abnormal in severe megaloblastic anemia.
- Marrow cells show erythroid hyperplasia with striking megaloblastic changes. Promegaloblasts with mitotic figures are abundant in severe cases. The number of sideroblasts is increased, and macrophage iron content may also be increased.
- Coexisting iron deficiency may reduce the megaloblastic erythroid morphologic changes, but hypersegmented neutrophils are still present in the blood, and giant metamyelocytes and bands persist in the marrow.
- Treatment of a patient with folic acid or cobalamin more than 12 hours before marrow biopsy may mask the megaloblastic changes.
- Serum bilirubin, iron, and ferritin levels are increased.
- Serum lactic dehydrogenase-1 and -2 and muramidase (lysozyme) levels are markedly elevated.
- See “Laboratory Diagnosis” below for measurement of cobalamin and folate tissue deficiency.

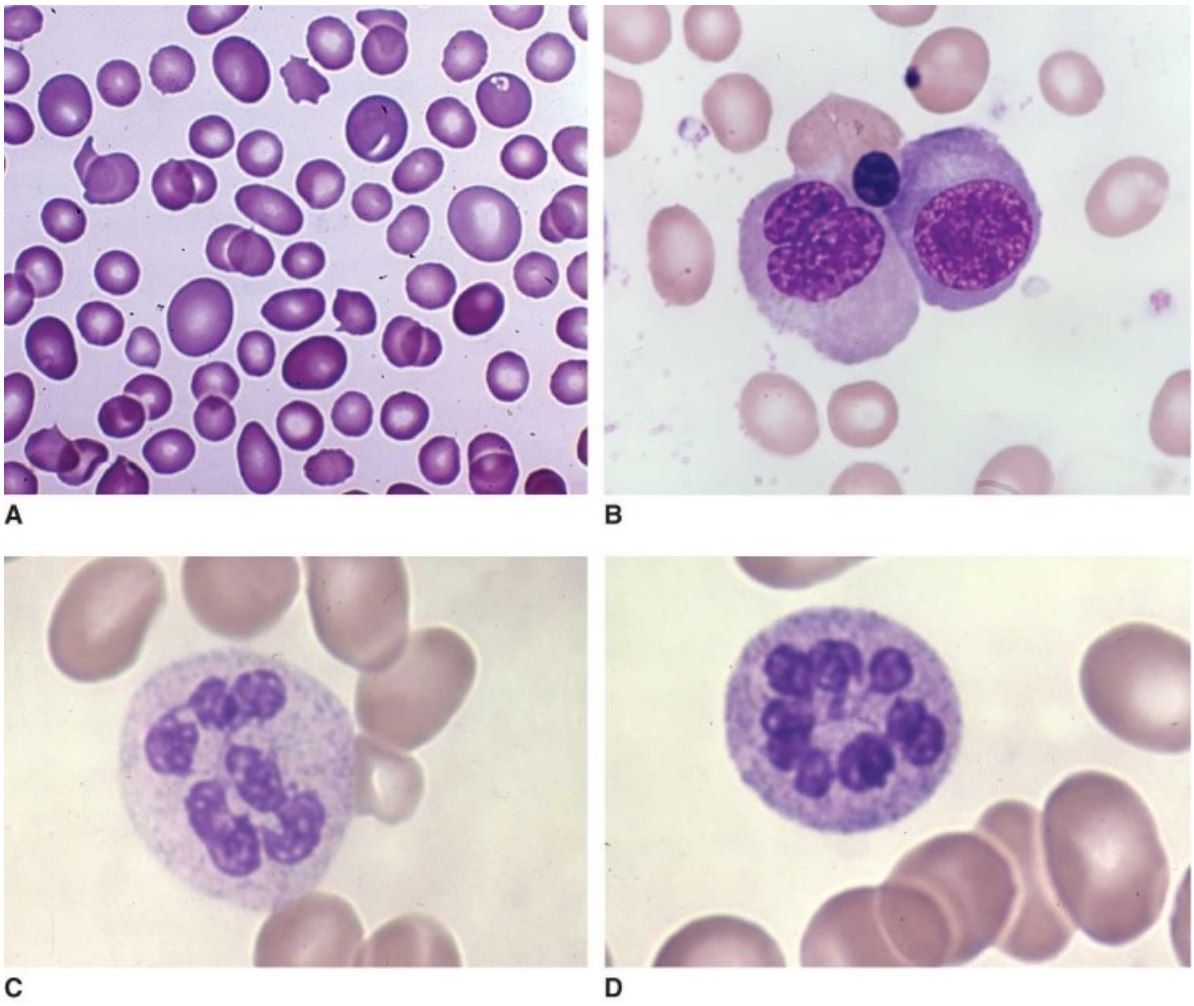


FIGURE 8–1 **A.** Pernicious anemia. Blood film. Note the striking oval macrocytes, wide variation in red cell size, and poikilocytes. Despite the anisocytosis and microcytes, the mean red cell volume is usually elevated, as in this case (121 fL). **B.** Marrow precursors in pernicious anemia. Note very large size of erythroblasts (megaloblasts) and asynchronous maturation. Cell on *right* is a polychromatophilic megaloblast with an immature nucleus for that stage of maturation. Cell on *left* is an orthochromatic megaloblast with a lobulated immature nucleus. An orthochromatic megaloblast with a condensed nucleus is between and above those two cells. **C** and **D.** Two examples of hypersegmented neutrophils characteristic of megaloblastic anemia. The morphology of blood and marrow cells in folate-deficient and vitamin B_{12}-deficient patients is identical. The extent of the morphologic changes in each case is related to the severity of the vitamin deficiency. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

DIFFERENTIAL DIAGNOSIS

- Macrocytosis occurs without megaloblastic anemia in patients with liver disease, hypothyroidism, aplastic anemia, myelodysplasia, pregnancy, and anemias with reticulocytosis, but in these settings, the MCV rarely exceeds 110 fL.
- Pancytopenia with reticulocytopenia, which is often present in severe megaloblastic anemia, should be distinguished from aplastic anemia (markedly hypocellular marrow without megaloblastic morphologic changes), myelodysplastic syndrome (often blasts in blood or marrow, dysmorphic neutrophils eg, acquired Pelger-Huet cells, hypogranular cells) and platelets (eg, abnormal size and granulation), and acute myelogenous leukemia (evident leukemic myeloblasts in marrow and usually blood).
- Certain chemotherapeutic drugs, especially folate antagonists (eg, methotrexate), hydroxyurea, and antiretroviral agents, may induce megaloblastic marrow and blood cell changes.

SPECIFIC FORMS OF MEGALOBLASTIC ANEMIA

Cobalamin Deficiency

- **Table 8–1** presents the causes of cobalamin deficiency.
- Cobalamin deficiency usually results from impaired absorption, most often as a consequence of a deficiency in gastric intrinsic factor (pernicious anemia).
- Less common causes include gastric and ileum resection syndromes, Zollinger-Ellison and "blind loop" syndromes, intestinal parasites, pancreatic disease, and dietary deficiencies.

Pernicious Anemia

- This disease of later life, usually after age 40 years, is caused by failure of secretion of intrinsic factor by the gastric mucosa.
- This form of anemia is an autoimmune disease in which there is immune destruction of the acid- and pepsin-secreting cells of the stomach.
- Antibodies to intrinsic factor are found in up to 70% of patients and are highly specific for pernicious anemia. Serum parietal cell antibodies are present in 90% of patients but are not specific.
- Concordance with several other autoimmune diseases (eg, immune thyroid diseases, type 1 diabetes mellitus, Addison disease, and others) is found.
- A family history is common, and dominant inheritance with low penetrance has been proposed. Pernicious anemia is more common in persons of Northern European or African descent and less common in those of Asian descent.
- Gastric atrophy and achlorhydria occur in all patients. Absence of achlorhydria is incompatible with diagnosis of pernicious anemia.
- The skin often assumes a lemon-yellow hue because of pallor combined with slight hyperbilirubinemia.
- Lingual papillary atrophy (smooth, beefy red tongue) is seen in advanced disease.
- The clinical features of cobalamin deficiency are those of megaloblastic anemia generally, plus neurologic abnormalities specifically caused by cobalamin deficiency.
- Neurologic abnormalities may occur before the onset of anemia and may be irreversible. The neurologic disorder usually begins with paresthesias of the fingers and toes and disturbances of vibration and position sense. The earliest signs may be loss of position sense in the second toe and loss of vibration sense to 256 Hz but not to 128 Hz. If untreated, the disorder progresses to spastic ataxia because of demyelination of the posterior and lateral columns of the spinal cord, referred to as *combined system disease*.
- Cobalamin deficiency also affects the brain, and patients may develop somnolence and perversion of taste, smell, and vision, sometimes with optic atrophy. Dementia or frank psychosis may occur, the latter sometimes referred to as "*megaloblastic madness*." Magnetic resonance imaging can confirm cobalamin deficiency affecting the brain by detecting demyelinization as T2-weighted hyperintensity of the white matter.
- Because neurologic complications may develop in patients with cobalamin deficiency treated with folic acid, a trial with folic acid is not recommended as a diagnostic test.
- Because of the possible development of neurologic complications in untreated patients with cobalamin deficiency, it is important to evaluate all patients with macrocytic anemia for both

cobalamin and folic acid deficiency.

Gastrectomy and Ileal Resection Syndromes

- Cobalamin deficiency develops within 5 to 6 years of total gastrectomy or resection of the terminal ileum as a result of loss of secretion of intrinsic factor from the stomach or failure to absorb cobalamin-intrinsic factor complexes in the ileum. The delay in onset of the anemia reflects the time required to exhaust cobalamin stores after absorption ceases. Diseases or injury to the terminal ileum may also lead to impaired cobalamin absorption and megaloblastic anemia (eg, regional ileitis, radiation, sprue).
- Cobalamin absorption may also be impaired after subtotal gastrectomy.

Zollinger-Ellison Syndrome

- Gastrin-secreting tumor, usually in the pancreas, stimulates gastric mucosa to elaborate immense amounts of hydrochloric acid.
- Sufficient acid may be secreted to inactivate pancreatic proteases and to prevent release of cobalamin from its binder, preventing its attachment to intrinsic factor; both are necessary for cobalamin absorption.

"Blind Loop" Syndrome

- Intestinal stasis from anatomic lesions or impaired motility may lead to intestinal colonization with bacteria that bind cobalamin before it can be absorbed.

Diphyllobothrium latum Infestation

- These intestinal parasites, usually ingested in raw fish, bind cobalamin and prevent absorption. Only about 3% of people infested with the parasites become anemic. It is most prevalent in the Baltic Sea region, Canada, and Alaska where raw or undercooked fish is consumed. Diagnosis is made by identification of tapeworm ova in the feces.

Pancreatic Disease

- Pancreatic exocrine insufficiency leads to deficiency of pancreatic proteases necessary for cobalamin absorption. Clinically significant deficiency of cobalamin is rare.

Dietary Cobalamin Deficiency

- This type of megaloblastic anemia occurs rarely, usually in strict vegetarians who also avoid dairy products and eggs ("vegans").
- Symptomatic cobalamin deficiency can take decades to appear because of enterohepatic reabsorption of cobalamin, conserving body stores.
- Breast-fed infants of vegan mothers may also develop cobalamin deficiency.

Folic Acid Deficiency

- **Table 8–1** summarizes the causes of folic acid deficiency.
- An inadequate diet is the principal cause of folic acid deficiency. Folic acid reserves are

small, and deficiency can develop rapidly.
- Alcohol use can depress absorption and serum folate levels and can accelerate the appearance of megaloblastic anemia in people with early folate deficiency.

Megaloblastic Anemia Caused by Drugs

- Table 8–2 presents a partial list of drugs that cause megaloblastic anemia.
- Methotrexate is almost structurally identical to folic acid and acts by inhibiting dihydrofolate reductase, the enzyme which reduces folic acid to the active, tetrahydro form. Methotrexate toxicity is treated with folinic acid, which is already fully reduced, and therefore can bypass the inhibited dihydrofolate reductase.

TABLE 8–2 DRUGS THAT CAUSE MEGALOBLASTIC ANEMIA

Agents	Comments
Antifolates	
Methotrexate	Very potent inhibitor of dihydrofolate reductase
Aminopterin	Treat overdose with folinic acid
Pyrimethamine	Much weaker than methotrexate and aminopterin
Trimethoprim	Treat with folinic acid or by withdrawing the drug
Sulfasalazine	Can cause acute megaloblastic anemia in susceptible patients, especially those with low folate stores
Chlorguanide (-Proguanil)	
Triamterene	Use of folate and cobalamin during pemetrexed treatment reduces toxicity
Pemetrexed (Alimta)	
Purine analogs	
6-Mercaptopurine	Megaloblastosis precedes hypoplasia, usually mild
6-Thioguanine	Responds to folinic acid but not folate
Azathioprine	
Acyclovir	Megaloblastosis at high doses
Pyrimidine analogs	
5-Fluorouracil	Mild megaloblastosis
Floxuridine (5′-fluorodeoxyuridine)	
6-Azauridine	Blocks uridine monophosphate production by inhibiting orotidyl decarboxylase; occasional megaloblastosis with orotic acid and orotidine in urine
Zidovudine (AZT)	Severe megaloblastic anemia is the major side effect
Ribonucleotide reductase inhibitors	
Hydroxyurea	Marked megaloblastosis within 1–2 days of starting therapy; quickly reversed by withdrawing drug
Cytarabine (cytosine arabinoside)	Early megaloblastosis is routine
Anticonvulsants	
Phenytoin (-diphenylhydantoin)	Occasional megaloblastosis, associated with low folate levels; responds to high-dose folate (1–5 mg/day); how anticonvulsants cause low folate is not understood but may be related to a drug-induced rise in cytochrome P450

Phenobarbital	
Primidone	
Carbamazepine	
Other drugs that depress folates	
Oral contraceptives	Occasional megaloblastosis; sometimes dysplasia of uterine cervix, corrected with folate
Glutethimide	
Cycloserine	
H+/K+-ATPase inhibitors	
Omeprazole	Long-term use causes decreased serum cobalamin levels
Lansoprazole	
Miscellaneous	
N_2O	See “Acute Megaloblastic Anemia”
p-Aminosalicylic acid	Causes cobalamin malabsorption with occasional mild megaloblastic anemia
Metformin	
Phenformin	Causes cobalamin malabsorption but not anemia
Colchicine	
Neomycin	
Arsenic	Causes myelodysplastic hematopoiesis, sometimes with megaloblastic changes

Source: *Williams Hematology,* 9th ed, Chap. 41, Table 41–5.

Acute Megaloblastic Anemia

- Acute megaloblastic anemia refers to a syndrome of rapidly developing thrombocytopenia and/or leukopenia, with very little change in the hemoglobin level. The marrow is floridly megaloblastic.
- The most common cause is nitrous oxide anesthesia. Nitrous oxide destroys methylcobalamin, inducing cobalamin deficiency. The marrow becomes megaloblastic within 12 to 24 hours. Hypersegmented neutrophils appear in the blood after 5 days.
- Serum cobalamin levels are low in most affected patients. Cobalamin levels are usually normal in cobalamin deficiency resulting from exposure to nitrous oxide and in some of the inherited abnormalities of cobalamin metabolism (see below).
- The effects of nitrous oxide disappear in a few days. Administration of folinic acid or cobalamin accelerates recovery.
- Fatal megaloblastic anemia has occurred in patients with tetanus who were treated with nitrous oxide for weeks.
- Acute megaloblastic anemia may also occur in seriously ill patients in intensive care units, patients transfused extensively, patients on dialysis or total parenteral nutrition, or patients receiving weak folic acid antagonists. The diagnosis is made from finding a megaloblastic marrow. Treatment is with both parenteral cobalamin (1 mg) and folic acid (5 mg).

Megaloblastic Anemia in Childhood

- Cobalamin malabsorption occurs in the presence of normal intrinsic factor in an inherited disorder of childhood (*selective malabsorption of cobalamin,* or *Imerslund-Graesbeck disease*). There is associated albuminuria. Anemia usually develops before age 2 years. Treatment is with parenteral cobalamin.
- *Congenital intrinsic factor deficiency* is an autosomal recessive disorder in which parietal cells fail to produce intrinsic factor. The disease presents at 6 to 24 months of age. Treatment is with parenteral cobalamin.
- *Transcobalamin II deficiency* is an autosomal recessive disorder that leads to megaloblastic anemia in early infancy. Serum cobalamin levels are normal, but there is severe tissue cobalamin deficiency because transcobalamin II mediates transport of cobalamins into the tissues. The diagnosis is made by measuring serum transcobalamin II concentration. Treatment is with sufficiently large doses of cobalamin to override the deficient transport.
- *True juvenile pernicious anemia* is an extremely rare disorder that usually presents in adolescence. The diagnosis and treatment are the same as for the adult disease.

Other Megaloblastic Anemias and Changes

- Megaloblastic anemia may occur in some patients with inborn errors of cobalamin metabolism, inborn errors of folate metabolism, hereditary orotic aciduria, and the Lesch-Nyhan syndrome. A thiamine responsive megaloblastic anemia has also been reported.
- Anemia with megaloblastic-like red cell morphology ("megaloblastoid") may occur in some patients with congenital dyserythropoietic anemias, myelodysplastic syndromes, and erythroleukemia.

LABORATORY DIAGNOSIS

Cobalamin Tissue Deficiency

- Serum cobalamin levels are low in most affected patients but may be normal because of nitrous oxide inhalation and some of the inherited abnormalities of cobalamin metabolism.
- Serum cobalamin levels may be low with normal tissue levels in vegetarians, older persons, the chronically ill, people taking megadoses of vitamin C, pregnancy (25%), transcobalamin I deficiency, or folate deficiency (30%).
- Transcobalamin-bound cobalamin represents about 25% of the total plasma cobalamin and is the functionally important fraction. Assays permit measurement of this more relevant, transcobalamin-bound cobalamin level.
- Methylmalonic aciduria and elevated serum levels of methylmalonic acid are reliable indicators of tissue cobalamin deficiency (except in the presence of severe renal insufficiency). They are the earliest changes and precede anemia or morphologic blood cell changes. If normal, they argue strongly against tissue deficiency even if serum levels of the vitamin are low.
- Elevated serum homocysteine can indicate tissue cobalamin deficiency but, unlike abnormalities in methylmalonic acid noted above, it can also be elevated in folic acid deficiency, pyridoxine deficiency, and hypothyroidism.
- In patients were pernicious anemia, serum intrinsic factor antibodies are present in 7% of

patients and are specific for the diagnosis.

Folic Acid Deficiency

- Serum folate levels are reduced, but a low level may merely reflect reduced oral intake in the few days preceding the test.
- The red cell folic acid level is a more accurate reflection of tissue folate because it is not affected by recent dietary intake or drugs.
- Both red cell and serum folate are decreased in folic acid deficiency. In cobalamin deficiency, red cell folate may be low but serum folate is normal or elevated. Thus, both measurements are required to assess tissue folate levels.

THERAPY, COURSE, AND PROGNOSIS

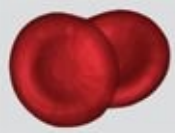
Cobalamin Deficiency

- Treatment consists of parenteral administration of cyanocobalamin (vitamin B_{12}) or hydroxycobalamin in doses sufficient to replete tissue stores and provide daily requirements.
- Vitamin B_{12} has no toxicity per se, but parenteral cobalamin doses larger than 100 µg saturate the transport proteins and much is lost in the urine.
- A typical treatment schedule consists of 1000 µg of vitamin B_{12} intramuscularly daily for 2 weeks, then weekly until the hemoglobin level is normal, and then monthly for life.
- It has been recommended that after initial therapy, to return the hematocrit to normal, patients with neurologic abnormalities should receive 1000 µg intramuscularly every 2 weeks for 6 months.
- About 1% of an oral dose of vitamin B_{12} is absorbed even in the absence of intrinsic factor. Therefore, patients with pernicious anemia can be successfully treated with oral vitamin B_{12} in doses of 1,000 µg/day. Patients receiving such therapy should be carefully monitored to ensure compliance and a response.
- Infection can interfere with the response to vitamin B_{12} therapy.
- Transfusion may be required if the clinical picture requires prompt alleviation of anemia. Most patients, however, have adapted to severe anemia and can be treated with vitamin replacement therapy.
- Following initiation of cobalamin therapy, there is often a prompt improvement in the sense of well being.
- Marrow erythropoiesis converts from megaloblastic to normoblastic beginning about 12 hours after treatment is started.
- Reticulocytosis appears on days 3 to 5 and reaches a peak on days 4 to 10. The hemoglobin concentration should become normal within 1 to 2 months.
- Leukocyte and platelet counts normalize promptly, although neutrophil hypersegmentation persists for 10 to 14 days.
- Elevated serum bilirubin, serum iron, and lactic dehydrogenase levels fall to normal rapidly.
- Severe hypokalemia may develop after cobalamin therapy, and death from hypokalemia has occurred. Potassium levels must be monitored and appropriate replacement given.

- Cobalamin therapy should be administered to all patients after total gastrectomy or resection of the terminal ileum. After partial gastrectomy, patients should be monitored carefully for the development of anemia.
- The anemia of the “blind loop” syndrome will respond to parenteral cobalamin therapy, but it also responds to oral antibiotic therapy or successful correction of an anatomic abnormality.
- Pregnant women at risk for cobalamin deficiency, such as strict vegetarians, may also be given vitamin B_{12}, 1 mg parenterally every 3 months, during pregnancy.

Folic Acid Deficiency

- Folic acid deficiency responds to physiologic doses of folic acid (200 µg/day), but cobalamin deficiency responds only to folic acid doses of 5 mg/day.
- Folic acid is administered orally at a dose of 1 to 5 mg daily. At this dosage, patients with malabsorption usually respond.
- Pregnant women should receive 1 mg of folic acid daily.
- In megaloblastic anemia with laboratory evidence of folic acid deficiency, a full response to physiologic doses of folic acid should occur. If a question of absorptive limitations is present, the folate should be administered intramuscularly.



For a more detailed discussion, see Ralph Green: Folate, Cobalamin, and Megaloblastic Anemias, Chap. 41 in *Williams Hematology*, 9th ed.

CHAPTER 9

Iron-Deficiency Anemia and Iron Overload

- Iron deficiency is one of the most common chronic maladies in humans. One third to one half of healthy females of reproductive age in the United States have absent iron stores, and 10% have iron-deficiency anemia, which is also common in infants and adolescents.
- Iron overload denotes an excess of iron in the body.

DEVELOPMENTAL STAGES OF IRON DEFICIENCY

- *Iron depletion:* storage iron decreased or absent
- *Iron deficiency:* storage iron absent with low serum iron concentration and transferrin saturation
- *Iron-deficiency anemia:* storage iron absent, low serum iron concentration and transferrin saturation, and low hemoglobin level

CAUSES OF IRON DEFICIENCY

- Chronic blood loss
- Diversion of maternal iron to fetus/infant during pregnancy/lactation
- Inadequate dietary intake of iron, primarily in infants and children
- Malabsorption of iron
- Intravascular hemolysis with hemoglobinuria and or hemosiderinuria
- Combinations of the above

Dietary Causes

- Infants most often develop iron deficiency because milk is a poor source of dietary iron and the additional requirements for iron imposed by rapid growth are not satisfied.
- In children, poor dietary intake plus intestinal parasites and/or bleeding gastrointestinal lesions are the usual causes.
- In the United States, average iron intake is 12 to 20 mg/d, varying by age and gender. Children and menstruating women are in precarious iron balance and at risk for iron deficiency.

Malabsorption

- Iron absorption is decreased in the malabsorption syndromes.
- After subtotal gastrectomy, malabsorption of dietary iron occurs in 50% of patients because of rapid gastrointestinal transit and because food bypasses the site of maximal absorption due to location of anastomosis. In contrast, medicinal iron is well absorbed after partial gastrectomy.

- In postgastrectomy anemia, there may be bleeding from anastomotic ulcer(s).

Chronic Blood Loss

- Menorrhagia is the most common cause of iron deficiency in women.
- Chronic blood loss may occur from the respiratory, gastrointestinal, or genitourinary tracts, or from phlebotomy for blood donation or laboratory testing, or may be self-induced.
- The most common cause of iron deficiency in men and postmenopausal women is gastrointestinal bleeding.

Pregnancy and Lactation

- The average iron loss from transfer to the fetus and blood in the placenta is 900 mg. Lactation losses of iron average 30 mg/mo.

PATHOGENESIS

- Lack of iron decreases heme synthesis, which leads to reduced hemoglobin synthesis and defective erythropoiesis.
- There is decreased activity of iron-containing enzymes, such as the cytochromes and succinic dehydrogenase.
- Neurologic dysfunction may occur, with impaired intellectual performance, paresthesias, and so on.
- Impaired performance during physical exertion is often present, especially in children and young adults.
- Atrophy of oral and gastrointestinal mucosa may occur, although this is unusual except in severe prolonged deficiency.
- In severe iron deficiency, gastric acid secretion may be reduced, often irreversibly.

CLINICAL FEATURES

- Patients develop the general symptoms of anemia (eg, easy fatigability, dyspnea on exertion, loss of sense of well-being).
- There is poor correlation between hemoglobin levels and severity of symptoms. Some patients with marked iron deficiency may deny the common symptoms of fatigue, weakness, or palpitations, whereas patients with mild iron deficiency may be symptomatic.
- Irritability and headache can occur.
- Children may have poor attention span, poor response to sensory stimuli, retarded developmental and behavioral achievement, and retarded longitudinal growth.
- Paresthesias and burning of the tongue may occur, possibly because of tissue iron deficiency.
- Pica, a craving to eat unusual substances such as clay or ice, is a classic manifestation, now most commonly reported by women of non-European ancestry.

PHYSICAL EXAMINATION

- Often unremarkable, especially when iron deficiency is mild
- Pallor, if anemia moderate or severe, best seen in the conjunctivae
- Smooth red tongue, stomatitis
- Angular cheilitis
- Koilonychia (rare and limited to severe chronic deficiency)
- Retinal hemorrhages/exudates (rare and limited to severe chronic deficiency)
- Splenomegaly (rare and limited to severe chronic deficiency)

LABORATORY FEATURES

Red Blood Cells

- Earliest change is anisocytosis and increased red cell distribution width (**Figure 9–1**), although at early stages in patients with minimal and even moderate anemia, these abnormalities can be absent.
- Mild ovalocytosis is present, with target cells.
- Progressive hypochromia (low mean cell hemoglobin concentration) and microcytosis (low mean cell volume [MCV]) occur.
- Reticulocytes are normal or reduced.
- Measurement of the reticulocyte hemoglobin content in a flow cytometer is a sensitive index of early iron deficiency.
- Soluble transferrin receptor is increased.

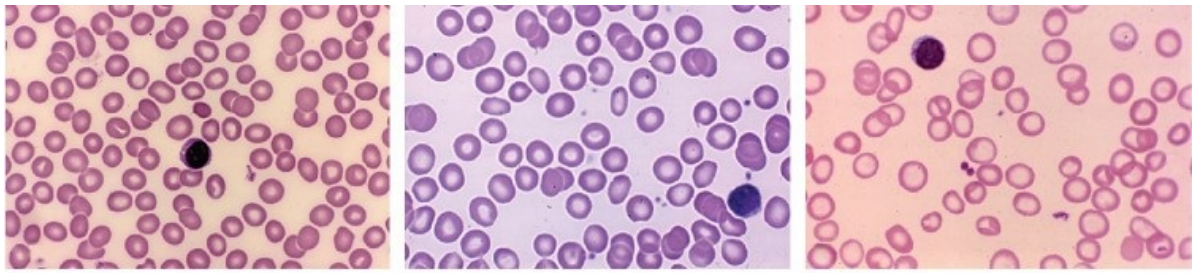


FIGURE 9–1 The characteristic hypochromia and microcytosis of moderately severe iron deficiency anemia. **A**. Normal blood film. **B**. Mild iron deficiency anemia. **C**. Severe iron deficiency anemia. Note advancing hypochromia and anisocytosis from **A** through **C**. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

Leukocytes

- Leukopenia (3–4.4 × 10^9/L) is found in a small number of patients. Differential white count is normal.

Platelets

- Thrombocytopenia develops in approximately one fourth of iron-deficient children but is very uncommon in adults.
- Thrombocytosis is found in approximately one third of iron-deficient children but is very common among adults and usually secondary to chronic active blood loss.

Marrow

- Marrow cellularity is normal and myeloid-to-erythroid ratio is variable.
- Sideroblasts are absent.
- There is markedly decreased to absent macrophage hemosiderin by Prussian blue staining.
- Erythroblasts may be small, with narrow rim of ragged cytoplasm and poor hemoglobin formation (micronormoblasts with defective hemoglobinization).

Serum Iron Concentration and Total Iron-Binding Capacity (TIBC)

- Serum iron concentration may be in the low-normal range in mild deficiency.
- TIBC is usually increased but may be in the high-normal range in mild deficiency.
- Saturation (iron/TIBC) is often 15% or less, but this is not specific for iron deficiency. It occurs also in chronic inflammation and severe inflammatory states (eg, arthritis, pericarditis, and others).
- Serum transferrin receptor level may be of utility when ferritin levels are borderline low. Its elevation provides further evidence for the diagnosis of an early stage of iron deficiency.

Serum Ferritin

- Levels of less than 10 µg/L are characteristic of iron deficiency.
- Levels of 10 to 20 µg/L are presumptive but not diagnostic.
- Values may be elevated with concomitant inflammatory diseases (eg, rheumatoid arthritis), Gaucher disease, chronic renal disease, malignancy, hepatitis, or iron administration.
- Iron deficiency can be suspected in rheumatoid arthritis or other severe inflammatory states if the ferritin level is less than 60 µg/L.

Reticulocyte Hemoglobin Content

- Reticulocyte hemoglobin content in a flow cytometer is a sensitive index of early iron deficiency. A value of less than 26 pg/cell is generally indicative of iron deficiency.

Soluble Transferrin Receptor

- Concentration is usually increased in iron deficiency.
- Serum transferrin receptor and its ratio to serum ferritin correlates well with depleted iron stores.

Free Erythrocyte Protoporphyrin (FEP)

- Concentration is usually increased in iron deficiency.
- FEP is a sensitive test for diagnosis of iron deficiency and suitable for large-scale screening of children, detecting both iron deficiency and lead poisoning.

DIAGNOSIS

- The physician who establishes a diagnosis of iron deficiency should assiduously search for a source of blood loss if unapparent or if another, much less common, reason for the deficiency

is not evident. Because the source of bleeding may represent a greater health threat than the iron deficiency itself, determination of the site and cause of the blood loss is essential.
- As in all deficiency states leading to anemia, the diagnostic findings depend on the severity of the deficiency. If early and mild, the expected changes (eg, decreased serum iron and percent saturation of transferrin) may be mild or within the normal range.

Special Studies

- Gastrointestinal loss is the most prevalent site of blood loss in men and uterine menstrual loss in women. Multiple stools should be tested for occult blood in every patient with iron deficiency.
  — Bleeding may be intermittent.
  — Common screening tests are insensitive to less than 5 to 10 mL blood loss per day.
  — Endoscopic and radiographic studies, including capsule endoscopy, may detect the source of gastrointestinal bleeding.
  — Angiography may be helpful if active bleeding is 0.5 mL/min or greater.
  — Pertechnetate uptake studies may detect a Meckel diverticulum.
- Hemosiderin-laden macrophages are in sputum if intrapulmonary bleeding is present.
- Urinary hemosiderin detection (iron-laden urine epithelial cells) is a definitive way of confirming iron deficiency due to intravascular hemolysis. The first morning urine is the most sensitive sample collection time for finding iron in the urine. Urinary iron loss can also be measured quantitatively by atomic absorption spectroscopy.

DIFFERENTIAL DIAGNOSIS

- Iron deficiency versus thalassemia versus anemia of chronic disease (Table 9–1)

TABLE 9–1 MICROCYTIC DISORDERS THAT MAY BE CONFUSED WITH IRON DEFICIENCY

Thalassemias and Hemoglobinopathies (see Chaps. 15 and 17)
β-Thalassemia major
β-Thalassemia minor
δβ-Thalassemia minor
α-Thalassemia minor
Hemoglobin Lepore trait
Hemoglobin E trait
Homozygous hemoglobin E disease
Hemoglobin H disease
Combination of above (compound heterozygotes)
Blockade of Heme Synthesis Caused by Chemicals (see Chaps. 11 and 20)
Isoniazid
Lead
Pyrazinamide
Sirolimus
Other Disorders
Anemia of chronic inflammation (see Chap. 5)
DMT-1 human mutations
Sideroblastic anemias (see Chap. 11)
Aceruloplasminemia
Atransferrinemia

Erythropoietic porphyrias
Hereditary sex-linked
Idiopathic acquired
STEAP3 deficiency

Source: *Williams Hematology,* 9th ed, Chap. 43, Table 43–2.

TREATMENT

Therapeutic Trial

- This should initially be by oral route, if possible.
- Response to expect is:
  — Peak reticulocytosis at approximately 10 days. However, reticulocyte response is a function of severity of anemia and may be modest in mild anemia
  — Significant increase in hemoglobin concentration at 3 to 4 weeks
  — Hemoglobin level normal at 1 to 4 months
- Unless there is continued bleeding, a coexisting inflammatory disease, or evidence for iron malabsorption, absence of a response indicates that iron deficiency is not the cause of anemia. Iron treatment should be stopped and another mechanism of anemia sought. (See also "Failure to Respond to Therapy," below.)

Oral Iron Therapy

- Dietary sources are insufficient for treatment.
- Safest, least expensive are oral ferrous salts (eg, ferrous sulfate or ferrous gluconate).
- Coated forms should never be used.
- Administration with meals or antacids or with inhibitors of acid production decreases efficacy.
- Daily total of 150 to 200 mg elemental iron in three to four doses, each 1 hour before meals (65 mg of elemental iron is contained in 325 mg of ferrous sulfate USP, or in 200 mg dried ferrous sulfate) is optimal.
- Some patients may complain of gastrointestinal intolerance to pills, pyrosis, constipation, diarrhea, and/or metallic taste and require:
  — Reduction of daily dose and frequency of administration
  — Change of oral iron preparation

Length of Treatment

- In order to replenish iron stores, continue oral therapy for 6 to 12 months after hemoglobin level is normal.
- Therapy may be needed indefinitely if bleeding continues (eg, menstruating women).

Parenteral Iron Therapy

- Routine use is usually not justified, unless used in renal failure patients on dialysis.
- Indications are:
  — Malabsorption

— Intolerance to oral iron preparations (colitis, enteritis)
— Need in excess of amount that can be given orally
— Preoperative autologous blood donation
— Patient uncooperative or unavailable for follow-up
— Renal failure patients on dialysis

- High-molecular-weight iron dextran (Dexferrum)
  — This is the first such agent available in the United States.
  — It can be given in higher single administration than other iron preparations.
  — Be aware of the danger of anaphylaxis or other systemic side effects (Food and Drug Administration [FDA] Black Box Warning).
  — Severe reactions are more common than with other parenteral preparations listed below.
  — This preparation contains 50 mg elemental iron per mL, with approximately 70% readily available for hemoglobin synthesis.
  — It may be given intramuscularly or intravenously.
  — See *Williams Hematology*, 9th ed, Chap. 43 or product information sheet for dosage calculations.
- Low-molecular-weight iron dextran (InFeD, CosmoFer)
  — This has a lower risk of drug reactions but still retains an FDA Black Box Warning.
- Iron sucrose (Venofer)
  — This is a complex of polynuclear iron ferric hydroxide in sucrose.
  — The manufacturer's recommended dose is 5 mL (100 mg of elemental iron).
  — Adverse events are reported by more than 5% of treated patients, with hypotension the most common.
- Ferric gluconate complex in sucrose (Ferrlecit)
  — This is a macromolecular complex of ferric iron.
  — The manufacturer recommends administration of doses of 125 mg of elemental iron with the preparation diluted in 100 mL of 0.9% sodium chloride and given intravenously over 1 hour.
  — Adverse events are reported by more than 5% of treated patients, with hypotension the most common.
- Ferumoxytol (Feraheme)
  — This intravenous iron preparation is approved for treatment of the anemia of chronic kidney disease.
  — It is a carbohydrate-coated, superparamagnetic iron oxide nanoparticle.
  — Clinical experience is limited. Approved in 2010, it received an FDA-mandated Black Box Warning in 2015 because of reports of serious reactions.

Failure to Respond to Therapy

- Wrong oral preparation (enteric-coated, insoluble iron, too little iron in each dose)
- Bleeding not controlled
- Therapy not long enough to show response
- Patient not taking medication
- Concomitant deficiencies (vitamin B_{12}, folate, thyroid hormone)
- Concomitant illness limiting erythropoietic response:

— Inflammation, infection, malignancy, hepatic disease, renal disease
- Diagnosis incorrect:
  — Thalassemia, lead poisoning, and so on

IRON OVERLOAD

Hereditary causes from inborn genetic errors and secondary acquired causes, such as those associated with inefficient erythropoiesis, hemolysis and transfusions are discussed here.

Etiology and Pathogenesis (see Table 9–2)

TABLE 9–2 CAUSES OF IRON OVERLOAD

Genetic	Acquired
Hereditary hemochromatosis (*HFE* mutation)	Chronic ingestion of medicinal iron
Juvenile hemochromatosis (*HAMP* or *HJV* mutations)	Transfusion iron overload
Hemochromatosis due to transferrin receptor 2 mutations	Acquired sideroblastic anemia
Ferroportin disease (*SLC40A1* mutations)	Siderosis associated with splenorenal or portocaval shunts
Neonatal hemochromatosis	
African hemochromatosis	
Thalassemia major (see Chap. 15)	
Hereditary sideroblastic anemia (see Chap. 11)	
Hereditary hemolytic anemias	
Enzyme deficiencies (see Chap. 15)	
Erythrocyte membrane disorders (see Chap. 13)	
Congenital dyserythropoietic anemias (see Chap. 7)	
Porphyria cutanea tarda (see Chap. 28)	
Hereditary atransferrinemia	
Hereditary aceruloplasminemia	

Source: *Williams Manual of Hematology*, 8th ed, Chap. 9, Table 9–2.

Classic Hemochromatosis (HFE hemochromatosis) (type 1)

- This diagnosis is applied to persons who have the hemochromatosis *HFE* genotype with increased body iron as suggested by increased serum ferritin levels, as well as to those who merely have the genotype, regardless of the level of their iron stores.
- This is an autosomal recessive disorder. Heterozygotes do not develop the disease.
- The major *HFE* mutation is cDNA nt 845 C → G (C282Y).
- The gene frequency is 0.06 to 0.08 in northern European populations so that about 15% of people are heterozygous and 0.5% are homozygous.
- It is very rare in non-Europeans.
- About one half of homozygotes have increased serum transferrin saturation and/or ferritin levels, but only 15% of homozygotes are clinically affected, and these are mostly males 40 to

60 years of age.
- Clinically significant disease is only seen in patients with serum ferritin higher than 1000. Thus clinical phenotype of hemochromatosis has a low penetrance and majority of homozygotes neither require nor benefit from therapy.
- A common minor mutation of *HFE* is cDNA nt 187 C → G (H63D).
- This gene's frequency is about 0.16 in the European population and is panethnic in its worldwide distribution.
- In the homozygous state (H63D/H63D), or in the compound heterozygous state (C282Y/H63D), this mutation may be also (but far less likely) associated with hemochromatosis.
- A number of "private" *HFE* mutations have been found in individual families.
- In affected individuals, excessive iron absorption through the gastrointestinal mucosa leads to accumulation of ferritin and hemosiderin in most cells in the body, especially in hepatocytes, with relative sparing of splenic macrophages.

Juvenile Hemochromatosis (type 2)
- The penetrance of the rare juvenile form of the disease is high, with onset of clinical disease during late adolescence and cardiomyopathy and endocrine deficiencies as the major clinical features.
- For list of mutations, refer to **Table 9–2**.

African Iron Overload
- African hemochromatosis is not caused by *HFE* or other known mutations.
- It is not clear to what extent African iron overload is a symptomatic condition.
- Many complicating factors, including malnutrition and high "home brewed" alcohol intake, are generally present.

Clinical Features
- Hereditary hemochromatosis usually presents in the fifth decade or later. High alcohol consumption increases the risk of serious manifestations. In the adult form, the male-to-female ratio is 5:1, and the disease is uncommon in premenopausal women.
- The juvenile forms of hereditary hemochromatosis presenting in children and young adults are very rare, affect both genders equally, and can present with multiendocrine failure and lethal cardiomyopathy.
- In adults, the most common symptoms are weakness, lethargy, loss of libido, joint symptoms, and weight loss.
- Arthralgia typically involves second and third metacarpophalangeal joints with swelling and tenderness. Hips and knees may also be involved, but because hemochromatosis is a disease of later life, a cause-and-effect relationship between joint symptoms and hemochromatosis is not established.
- Chondrocalcinosis or calcification of periarticular ligaments is a frequent late manifestation. Synovial fluid may contain calcium pyrophosphate and apatite crystals.
- Skin becomes hyperpigmented primarily from deposition of melanin.
- Cardiac effects are common.

— Arrhythmias
— Cardiomegaly (may be a result of restrictive or dilated cardiomyopathy)
— Congestive failure

- Endocrinopathies are common.
  — Pancreas (may show diffuse fibrosis and loss of islets). Diabetes mellitus occurs in some patients
  — Hypothyroidism (10% of male patients)
  — Hypothalamic pituitary insufficiency, usually involving gonadotropins (about half of patients)
  — Testicular atrophy, azoospermia, reduced libido, and impotence
  — Premature menopause
- Hepatomegaly and liver injury are common.
  — Jaundice is uncommon.
  — Liver function tests may be abnormal.
  — Hepatocellular carcinoma occurs in one third of patients who have fully developed disease.
  — Patients who have hemochromatosis and also consume significant quantities of alcohol are more likely to develop hepatic fibrosis than are patients who abstain from alcohol.
  — Cirrhosis found only in patients who have serum ferritin levels of greater than 1000 ng/mL and who have abnormal liver function tests.
- Splenomegaly is frequently present.

Laboratory Features

- The transferrin saturation exceeds 50% in one half of homozygotes for the C282Y mutation. The serum ferritin is greater than 200 ng/mL in one half of homozygous women and greater than 250 ng/mL in one half of homozygous men.
- Patients with fully developed disease may have hyperglycemia and an abnormal glucose tolerance test.
- Serum aminotransferase activities are increased in about 5% to 10% of homozygotes and in all of those with cirrhosis.
- Serum concentrations of pituitary gonadotropins and androgens are often low.
- Serum thyroxine levels may be low and thyroid-stimulating hormone concentrations increased.

Diagnosis

- Early diagnosis of clinically affected patients is imperative because body iron reduction therapy is relatively simple and prevents tissue injury, and complications may be irreversible and lead to death, for example from hepatocellular carcinoma.
- Serum ferritin levels are useful in screening but may be less sensitive than the serum iron level and transferrin saturation for screening but helpful to discriminate affected (ferritin > 1000 ng/mL) from asymptomatic patients.
- Estimates of the amount of marrow iron have little or no diagnostic value.
- Computed tomography scan and magnetic resonance imaging can demonstrate increased iron content of the liver.

- Liver biopsy is occasionally useful but is not required for diagnosis in most cases.
- In normal liver, iron content is less than 2.8 mg/g dry weight (50 µmol/g).
- In alcoholic liver disease, iron content is less than 5.6 mg/g (100 µmol/g).
- In hemochromatosis, iron content exceeds 5.6 mg/g (100 µmol/g), and in hemochromatosis with cirrhosis, iron content is usually greater than 11 mg/g (200 µmol/g).
- Genetic analysis for *HFE* mutations may be useful in family studies in order to detect other affected individuals.

Treatment

- Phlebotomy
  - — Removal of 500 mL blood by venesection every 1 or 2 weeks depletes the body iron burden. Each 500 mL removes about 200 mg of iron.
  - — The patient with severe disease usually has accumulated 30 to 40 g of excess iron.
  - — Adequacy of treatment may be gauged by progressive fall in hemoglobin level and the MCV, indicating a decrease of body iron below normal.
  - — For maintenance, removal of 500 mL blood every few months is usually sufficient. Serial measurements of serum ferritin levels are more useful than estimates of transferrin saturation in monitoring the effects of phlebotomy and should be used to monitor the patient indefinitely, with the goal of maintaining serum ferritin at less than 100 ng/mL.
  - — Alcohol and other hepatotoxins should be avoided.
- Oral chelating agents
  - — These are more expensive and have more side effects compared to phlebotomies.
  - — They may be useful when phlebotomies cannot be used or in acute life-threatening disease when rapid iron depletion is needed.
  - — For details, refer to Chap. 43, pp. 627-650, *Williams Hematology,* 9th ed.

Prognosis

- Life span is normal in patients without cirrhosis.
- Incidence of hepatocellular carcinoma is not diminished by treatment once there is hepatic fibrosis.
- Treatment may improve diabetes, cardiac function, and gonadal insufficiency.

SECONDARY HEMOCHROMATOSIS

Etiology and Pathogenesis

- Hyperabsorption of iron occurs in many hemolytic anemias, particularly in those accompanied by hyperactive (chronic hemolysis) and ineffective erythropoiesis (thalassemia).
- Blood transfusion adds to the iron burden and accelerates the clinical course.
- The administration of medicinal iron in the mistaken belief that iron will help the anemia is sometimes an aggravating factor.

Clinical Features

- These are the same as in hereditary hemochromatosis but in transfused patients, cardiac

manifestations frequently dominate.

Laboratory Features

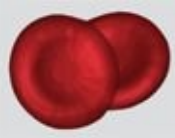
- Those of the underlying disease
- Elevated serum ferritin and transferrin saturation

Diagnosis

- In transfused patients, the iron burden can be estimated from the number of transfusions received (1 U contains ~200 mg iron), because daily iron loss is very small (~0.5–1.0 mg/dL).
- Elevation of ferritin levels reflects iron burden unless confounded by coexisting inflammation or hepatic injury.

Treatment

- Continuous subcutaneous deferoxamine infusion of 20 to 40 mg/kg/day, using a portable pump, remains the standard of care.
- Two oral iron chelators, deferiprone (25–33 mg/kg three times a day) and deferasirox (10–20 mg/kg once a day), are increasingly used alone or in combination with deferoxamine.
- Combinations of parenteral and oral agents are effective when adjusted for each patient based on iron burden and degree of cardiac involvement, patient compliance and side effects.



For a more detailed discussion, see Tomas Ganz: Iron Deficiency and Overload, Chap. 43 in *Williams Hematology*, 9th ed.

CHAPTER 10

Anemia Resulting from Other Nutritional Deficiencies

VITAMIN A DEFICIENCY

- This type of anemia is prevalent in school children in several underdeveloped African countries (eg, Malawi).
- Anemia is characterized by reduced mean (red) cell volume (MCV), mean (red) cell hemoglobin concentration, and anisocytosis and poikilocytosis.
- Unlike iron deficiency, but similar to the anemia of chronic disease, serum iron concentration is decreased, serum total iron-binding capacity is normal or low, and iron stores, reflected in serum ferritin levels, are increased. The anemia fails to respond to treatment with medicinal iron.
- The condition responds to vitamin A repletion.
- Coupling vitamin A with iron administration may lead to a faster response because of the evidence that vitamin A deficiency impairs iron utilization.

VITAMIN B_6 DEFICIENCY

Vitamin B_6 includes pyridoxal, pyridoxine, and pyridoxamine.

- Deficiency may lead to hypochromic microcytic anemia.
- Microcytic anemia may occur in patients taking isoniazid, which interferes with vitamin B_6 metabolism. Such an anemia may be corrected with large doses of pyridoxine.
- A small fraction of patients (5%–10%) who are not vitamin B_6 deficient may have sideroblastic anemia that will respond partially to high doses of pyridoxine (see Chap. 11).
- Malabsorptive states and renal dialysis may result in vitamin B_6 deficiency.

RIBOFLAVIN DEFICIENCY

- Volunteers receiving a riboflavin-deficient diet plus a riboflavin antagonist (galactoflavin) develop vacuolated erythroid precursors, followed by pure red cell aplasia—all reversed by administration of riboflavin.
- Reduced erythrocyte glutathione reductase activity occurs in riboflavin deficiency but is not associated with hemolysis or oxidant-induced injury.

THIAMINE DEFICIENCY

- Rare childhood syndrome is marked by diabetes mellitus, sensorineural deafness, and

megaloblastic anemia and occasionally thrombocytopenia.
- It is observed in children of Asian descent and results from mutation in *SLC19A2* gene on chromosome 1q23.3.
- It responds to lifelong administration of thiamine (25–100 mg/day).

VITAMIN C (ASCORBIC ACID) DEFICIENCY

- Anemia in humans with scurvy may be macrocytic, normocytic, or microcytic, and the marrow may be hypocellular, normocellular, or hypercellular. In approximately 10% of patients, the marrow hematopoiesis is megaloblastic.
- Macrocytic (megaloblastic) anemia may develop with vitamin C deficiency because vitamin C interacts with folic acid in the generation of tetrahydrofolic acid.
- Microcytic anemia may develop because vitamin C facilitates the absorption of iron and because of the bleeding manifestation of scurvy.
- Iron deficiency in children is often associated with dietary vitamin C deficiency.
- Normocytic normochromic anemia with a reticulocytosis of 5% to 10% also can develop as a manifestation of scurvy, perhaps from compromised cellular antioxidant defense mechanisms.
- The anemia of vitamin C deficiency responds promptly to administration of vitamin C. Sufficient folic acid and iron is required for the response to occur.
- If the anemia is macrocytic (megaloblastic), folate should be administered with vitamin C to obtain a timely response.

VITAMIN E (α-TOCOPHEROL) DEFICIENCY

- The vitamin E requirement varies with polyunsaturated fatty acid content of diet and the content of lipids that can peroxidize in tissues.
- Low-birth-weight infants often have low serum and tissue concentrations of vitamin E.
- A diet rich in polyunsaturated fatty acids and adequate in iron but inadequate in vitamin E may lead to hemolytic anemia by 4 to 6 weeks of age.
- Anemia is often associated with altered red cell morphology, thrombocytosis, and edema of the dorsum of the feet and pretibial area.
- These abnormalities are reversed promptly by treatment with vitamin E.
- Chronic fat malabsorption, such as is common in cystic fibrosis, can lead to vitamin E deficiency, if daily supplements of the water-soluble form of this vitamin are not given. In such patients, the red cell life span is mildly reduced and anemia may develop.
- Patients with sickle cell disease often have low serum vitamin E concentrations. Vitamin E deficiency has been associated with an increase in irreversibly sickled cells in the blood. Vitamin E (450 units/day) has been associated with a decrease in irreversibly sickled cells.

COPPER DEFICIENCY

- Copper is required for absorption and utilization of iron, perhaps functioning by maintaining iron in the ferric state for transferrin transport.

- Copper deficiency occurs in malnourished children and in infants and adults receiving parenteral alimentation and can also be caused by chronic ingestion of massive quantities of zinc, which impairs copper absorption.
- Young children with copper deficiency may have osteoporosis, flaring of ribs, and other bony abnormalities.
- Copper deficiency causes a microcytic anemia with hypoferremia, neutropenia, and vacuolated erythroid precursors in marrow that does not respond to iron therapy.
- Copper deficiency can occur after gastric resection or after bariatric gastric reduction surgery. The macrocytic anemia, neutropenia, and ringed sideroblasts in the marrow can mimic closely the clonal sideroblastic anemia seen in the myelodysplastic syndrome.
- Copper deficiency can be associated with secondary neurologic abnormalities, especially myeloneuropathy. Anemia in this situation can mimic cobalamin deficiency and should be considered in the differential diagnosis of the latter, especially in individuals postgastrectomy.
- Diagnosis is established by demonstration of low serum ceruloplasmin or copper levels, or by a therapeutic trial with copper at a dose of 0.2 mg/kg per day, orally. Copper levels are the more reliable measurement because ceruloplasmin is an acute phase reactant. A 10% solution of copper sulfate contains 25 mg of copper per milliliter.
- In copper deficiency related to gastric resection or bariatric surgery, therapy can be given with intravenous copper, 2.4 mg daily for 6 days, followed by weekly intravenous copper at the same dose with concomitant oral supplementation with copper gluconate, two 2.0-mg tablets taken twice daily (total of 8 mg/d of oral copper gluconate). Intravenous copper can be discontinued when patient without signs related to copper deficiency and maintained on oral copper. Continuous monitoring should be done to ensure adequate serum copper levels.
- Low serum copper values may also be seen in hypoproteinemic states (exudative enteropathies, nephrosis) and Wilson disease (see Chap. 20).

ZINC DEFICIENCY

- This condition may accompany thalassemia or sickle cell disease.
- Isolated zinc deficiency does not produce anemia.
- Zinc deficiency can result in growth retardation in children, impaired wound healing, impaired taste perception, and immunologic inadequacies.
- **Table 10–1** contains the normal levels in blood for the vitamins and minerals discussed above.

TABLE 10–1 RELEVANT BLOOD VITAMIN AND MINERAL LEVELS (ADULT VALUES)

Vitamin or Mineral	Serum Level	Plasma Level	Red Cell Level	White Cell Level
Copper	11–24 µmol/L		14–24 µmol/L	
Folate	7–45 nmol/L		> 320 nmol/L	
Riboflavin (B_2)	110–640 nmol/L		265–1350 nmol/L	
Vitamin A	1–3 µmol/L			
Vitamin B_6		20–122 nmol/L		
Vitamin C		25–85 µmol/L		11–30 attomol/cell
Vitamin E	12–40 µmol/L			

Selenium	1200–2000 nmol/L
Zinc	11–18 µmol/L

Source: *Williams Hematology,* 9th ed, Chap. 44, Table 44–1.

ANEMIA OF STARVATION

- Semistarvation causes mild to moderate normocytic normochromic anemia with reduced marrow erythroid precursors. The anemia is principally dilutional.
- Complete starvation for 9 to 12 weeks leads to anemia and marrow hypocellularity, which responds to resumption of a normal diet. The decreased hemoglobin may be a response to a hypometabolic state with consequent decrease in oxygen requirements. Reticulocytosis and correction of the hemoglobin deficit follows refeeding.

ANEMIA OF PROTEIN DEFICIENCY (KWASHIORKOR)

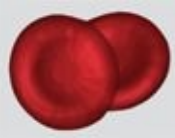
- In protein-calorie malnutrition, the hemoglobin level may fall to 8 g/dL, but some children may not be as anemic because the reduced red cell mass is masked by a reduced plasma volume.
- Anemia is normocytic, normochromic, with significant anisocytosis and poikilocytosis.
- Leukocyte and platelet counts are usually normal.
- The marrow is usually normocellular or hypocellular with reduced erythroid precursors.
- Patients respond slowly to high-protein diets (powdered milk or essential amino acids).
- After 3 or 4 weeks of treatment, there may be an episode of erythroid aplasia that responds to riboflavin or prednisone.
- Occult deficiencies may become manifest during the repletion period (eg, iron, folic acid, vitamin E, and vitamin B_{12}).

ALCOHOLISM

- Chronic alcohol ingestion is often associated with anemia, which may be a result of multiple causes:
  — Nutritional deficiencies
  — Chronic gastrointestinal bleeding
  — Hepatic dysfunction
  — Hemolytic anemia
  — Hypersplenism from portal hypertension
  — Direct toxic effects of ethanol on erythropoiesis (and thrombopoiesis) and on folate metabolism
- Macrocytic anemia occurs commonly in hospitalized alcoholic patients and is often associated with megaloblastic changes and sometimes with ringed sideroblasts.
- Megaloblastic anemia in alcoholism is almost always caused by folic acid deficiency.
- Megaloblastic anemia is more common in drinkers of wine or whiskey, which have low folate

content, than in drinkers of beer, which is a rich source of folate.

- Alcoholics may have associated iron deficiency, producing a “dimorphic” blood picture (macrocytes, hypersegmented neutrophils, and hypochromic microcytes) and this may minimize an increase in MCV.
- Iron deficiency may be unmasked after treatment with folic acid alone by demonstration of an emerging population of microcytic red cells. Treatment with iron alone may unmask folate deficiency, by demonstration of an emerging population of macrocytes.
- Mild macrocytosis (MCV, 100–110 fL) is found in approximately 90% of chronic alcoholics. Anemia is usually absent, macrocytes are typically round as opposed to oval, in contrast to the red cells in megaloblastic anemia, and neutrophil hypersegmentation is not present. These patients are not folate deficient, and the macrocytosis persists until the patient abstains from alcohol.
- Concomitant thrombocytopenia may also be the result of the effects of alcohol.
- Hemolytic anemias that may occur include Zieve syndrome (alcohol-induced liver disease, hyperlipidemia, jaundice, and spherocytic hemolytic anemia) and spur cell anemia (severe alcohol-induced liver disease with hemolytic anemia and acanthocytes).



For a more detailed discussion, see Ralph Green: Anemia Resulting From Other Nutritional Deficiencies, Chap. 44 in *Williams Hematology*, 9th ed.

CHAPTER 11
Hereditary and Acquired Sideroblastic Anemias

- Sideroblastic anemias may be acquired or hereditary and are classified in **Table 11–1**.
- Normal red cell precursors have cytoplasmic organelles termed *siderosomes* that contain aggregated iron-rich ferritin. They can be seen in erythroblasts by transmission electron microscopy and represent normal structures providing iron for hemoglobin synthesis. These aggregates may be below the resolution of the light microscope. Thus, in Prussian blue–stained marrow specimens, about 20% to 40% of red cell precursors have one to three very small, pinhead-sized blue granules in the cytoplasm under oil immersion optics, depending on the quality of the preparation.
- Pathologic sideroblasts are of two types. The classical type is a *ringed sideroblast* with large, Prussian blue–stained granules in a circumferential position around the nucleus of the erythroblast. This position reflects their intramitochondrial location: mitochondria in erythroblasts being positioned closely surrounding the nucleus. The other type of pathologic sideroblast has large and multiple cytoplasmic granules (see **Figure 11–1**).
- Sideroblastic anemias are characterized by:
  — Commonly a population of hypochromic erythrocytes in the blood film (dimorphic picture)
  — Increased red cell precursors in the marrow in the face of anemia and a low reticulocyte count
  — Anemia that is the result of apoptosis of late erythroid precursors (ie, ineffective erythropoiesis), with increased plasma iron turnover and normal to decreased red cell survival
  — Drugs that reduce the formation of pyridoxal 5′-phosphate from pyridoxine decrease heme synthesis and can cause sideroblastic anemia

TABLE 11–1 CLASSIFICATION OF SIDEROBLASTIC ANEMIAS

I. **Acquired**
  A. Primary sideroblastic anemia (myelodysplastic syndromes) (see Chap. 44).
  B. Sideroblastic anemia secondary to:
    1. Isoniazid
    2. Pyrazinamide
    3. Cycloserine
    4. Chloramphenicol
    5. Ethanol
    6. Lead
    7. Chronic neoplastic disease
    8. Zinc-induced copper deficiency
    9. copper deficiency from malabsorption.

II. **Hereditary**
  A. X chromosome–linked
    1. *ALAS2* deficiency
    2. Hereditary sideroblastic anemia with ataxia: *ABCB7* mutations

B. Autosomal
   1. Defects in the erythroid specific mitochondrial carrier family protein *SLC25A38*
   2. Mitochondrial myopathy and sideroblastic anemia (*PSU1* mutations)

C. Mitochondrial: Pearson marrow-pancreas syndrome

Source: *Williams Hematology*, 9th ed, Chap. 59, Table 59–1.

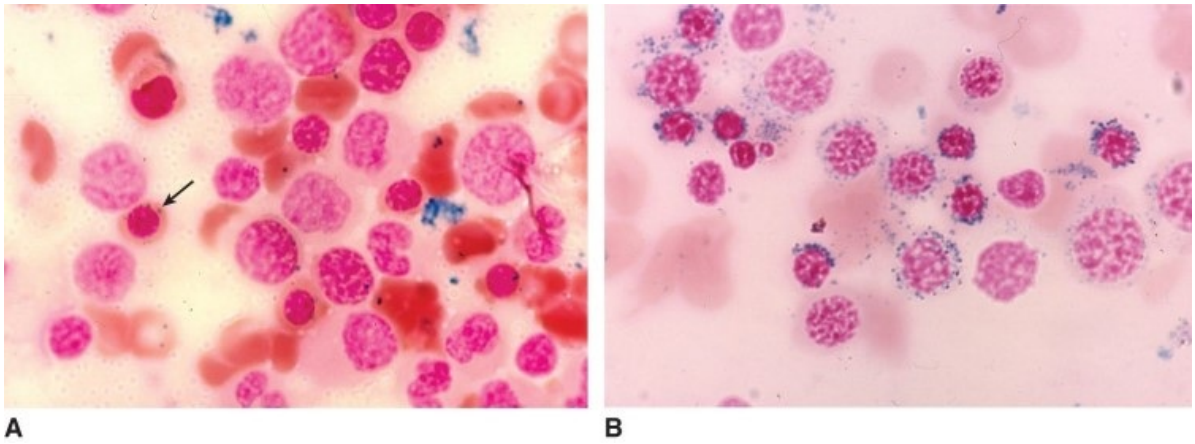


FIGURE 11–1 Marrow films. **A.** Normal marrow stained with Prussian blue. Note several erythroblasts without apparent siderotic (blue-stained) granules. The *arrow* indicates erythroblast with several very small cytoplasmic blue-stained granules. It is very difficult to see siderosomes in most erythroblasts in normal marrow because they are often below the resolution of the light microscope. **B.** Sideroblastic anemia. Note the florid increase in Prussian blue staining granules in the erythroblasts, most with circumnuclear locations. These are classic examples of ringed sideroblast that are by definition pathologic changes in the red cell precursors. In some cases, cytoplasmic iron granules are also increased in size and number, also a pathologic change. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

ACQUIRED SIDEROBLASTIC ANEMIA

Primary

- There is a clonal (neoplastic) anemia with varying frequencies of neutropenia and thrombocytopenia or less commonly thrombocytosis. This feature of the myelodysplastic syndromes is discussed in Chap. 44.

Secondary

- The anemia is most commonly associated with use of isonicotinic acid hydrazide, pyrazinamide, or cycloserine.
- It is common in the marrow of alcoholics and a diagnostic feature of the anemia of alcohol abuse.
  - In the anemia of chronic alcoholism, folate deficiency may coexist as a result of inadequate diet. Thus, the anemia may have megaloblastic features and ringed sideroblasts. On removal of alcohol and replacement of folate, the megaloblastic features disappear first, and the sideroblastic features disappear at a later time as long as abstinence from alcohol is in place.
- Anemia may occur in patients with neoplastic or chronic inflammatory diseases.
- Anemia may be severe and is characterized by dimorphic red cells on the blood film, hypochromic and normochromic.
- If drugs are responsible, the anemia responds promptly to withdrawal of the offending agent.

- In cases related to an underlying disease, improvement is associated with successful treatment of the primary disease.

HEREDITARY SIDEROBLASTIC ANEMIA

Inheritance

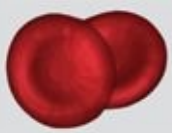
- The X-linked form is a result of mutations of erythroid-specific ALA synthase (*ALAS2*).
- Some autosomal forms have also been described.
- A mitochondrial deletion causes *Pearson marrow-pancreas syndrome*.
  — This condition is generally not inherited but arises from de novo mutations that likely occur in early embryonic development.

Clinical and Laboratory Manifestations

- Anemia appears in the first few weeks or months of life.
- It is characteristically microcytic and hypochromic.
- There is prominent red cell dimorphism, with striking anisocytosis and poikilocytosis.
- Splenomegaly is usually present.
- Iron overload develops frequently.

Treatment

- Patients with hereditary sideroblastic anemia may respond to pyridoxine in oral doses of 50 to 200 mg daily.
- Folic acid administered concomitantly may increase the response.
- Full normalization of the hemoglobin level is usually not achieved, and relapse occurs if pyridoxine therapy is stopped.
- Efforts should be made to reduce iron overloading by phlebotomy, if possible when anemia is mild or by iron chelators, if phlebotomy is not tolerated (see Chap. 9).



For a more detailed discussion, see Prem Ponka and Josef T. Prchal: Hereditary and Acquired Sideroblastic Anemias, Chap. 5 in *Williams Hematology*, 9th ed.

CHAPTER 12
Anemia Resulting from Marrow Infiltration

DEFINITIONS

- Anemia or pancytopenia associated with extensive marrow infiltration is called *myelophthisic anemia*.
- *Leukoerythroblastosis* refers to the presence of nucleated red cells, and myeloid precursor cells (eg, neutrophilic myelocytes) in the blood. These finding may be accompanied by schistocytes, teardrop-shaped red cells, and megakaryocytic fragments in patients with myelophthisic anemia.

ETIOLOGY AND PATHOGENESIS

- Table 12–1 lists the conditions that cause marrow infiltration.
- Invasion of blood vessels is the essential component of cancer cell metastasis and often involves the loss of E-cadherin.
- In most cases, the marrow infiltration of metastatic cells is focal, with surrounding areas of normal or hyperactive marrow.
- Disruption of the microenvironment by infiltration with foreign cells leads to premature release of immature blood cells from the marrow.
- Myelophthisic anemia is most often caused by humoral factors (eg, cytokines) and injury to the marrow microenvironment.

TABLE 12–1 CAUSES OF MARROW INFILTRATION

I. **Fibroblasts and Collagen** A. Primary myelofibrosis (see Chap. 47) B. Fibrosis of other myeloproliferative disorders (see Chaps. 45 and 46) C. Fibrosis of hairy cell leukemia (see Chap. 56) D. Metastatic malignancies (eg, breast carcinoma) E. Sarcoidosis F. Secondary myelofibrosis with pulmonary hypertension
II. **Other Noncellular Material: oxalosis**
III. **Tumor Cells** A. Carcinoma (eg, lung, breast, prostate, kidney, thyroid and neuroblastoma) B. Sarcoma
IV. **Granulomas (inflammatory cells)** A. Miliary tuberculosis B. Fungal infections C. Sarcoidosis
V. **Macrophages** A. Gaucher disease (see Chap. 37)

B. Niemann-Pick disease (see Chap. 37)
C. Macrophage activation syndrome (MAS)

VI. **Marrow Necrosis**
A. Sickle cell anemia (see Chap. 16)
B. Solid tumor metastasis
C. Septicemia
D. Acute lymphoblastic leukemia
E. Arsenic therapy

VII. **Failure of Osteoclast Development: osteopetrosis**

Source: *Williams Hematology,* 9th ed, Chap. 45, Table 45–1.

CLINICAL FEATURES

- The clinical features of marrow infiltrative disorders are usually those of the underlying disease, but the marrow replacement may also accentuate associated cytopenias.

LABORATORY FEATURES

- Anemia is mild to moderate.
- Leukocyte and platelet counts may be high or low depending on the nature and extent of marrow replacement.
- Blood film may show anisocytosis and poikilocytosis, with schistocytes, teardrop cells, nucleated red cells, immature granulocytic cells, and megakaryocytic fragments.
- Leukocyte alkaline phosphatase activity is normal or increased.
- Clusters of cancer cells rarely may be found on the blood film (carcinocythemia).
- Marrow biopsy is the most reliable diagnostic procedure. Marrow aspiration may also be of value. Aspiration or biopsy is more likely to be positive if taken from a tender area of bone.
- Sites of marrow infiltration may be detected by technetium-99m sestamibi uptake, magnetic resonance imaging, or fluorine-18 fluorodeoxyglucose with positron emission tomography.

DIFFERENTIAL DIAGNOSIS

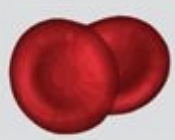
- Nucleated red cells and leukocytosis can be seen in overwhelming sepsis, primary myelofibrosis, acute severe hypoxia (eg, acute congestive heart failure), thalassemia major, and severe hemolytic anemia.
- Primary myelofibrosis (see Chap. 47) may be confused with metastatic disease with focal fibrosis.
- In the absence of a known primary site of cancer, it is important to rule out sarcoma of bone.

TREATMENT AND PROGNOSIS

- The goal of treatment is to manage the underlying disease.
- Marrow infiltration may not always adversely affect the response to treatment of malignant

disease.

- However, usually short-term survival is seen in patients with cancer metastatic to marrow. Patients with breast and prostate cancer metastatic to marrow have longer survival on average than those with lung cancer.



For a more detailed discussion, see Vishnu Reddy and Josef T. Prchal: Anemia Associated with Marrow Infiltration, Chap. 45 in *Williams Hematology*, 9th ed.

CHAPTER 13

Erythrocyte Membrane Disorders

THE ERYTHROCYTE MEMBRANE

- The erythrocyte membrane plays a critical role in the maintenance of the biconcave shape and integrity of the red cell.
- It provides flexibility, durability, and tensile strength, enabling erythrocytes to undergo extensive and repeated distortion during their passage through the microvasculature.
- It consists of a lipid bilayer with embedded transmembrane proteins and an underlying membrane protein skeleton that is attached to the bilayer via linker proteins.
- The integrity of the membrane relies on *vertical* interactions between the skeleton and the bilayer, as well as on *horizontal* interactions within the membrane skeletal network.

ERYTHROCYTE MEMBRANE ABNORMALITIES

- Inherited membrane protein defects disrupt the membrane architecture and alter the shape of the cell resulting in hemolytic anemia as illustrated in **Figure 13–1**.
- Protein defects that compromise *vertical* interactions between the membrane skeleton and the lipid bilayer result in destabilization of the bilayer, loss of membrane microvesicles, and *spherocyte* formation.
- Protein defects affecting *horizontal* protein interactions within the membrane skeletal network disrupt the skeleton, resulting in defective shape recovery and *elliptocytes*.
- Red cell membrane disorders exhibit significant heterogeneity in their clinical, morphologic, laboratory, and molecular characteristics.

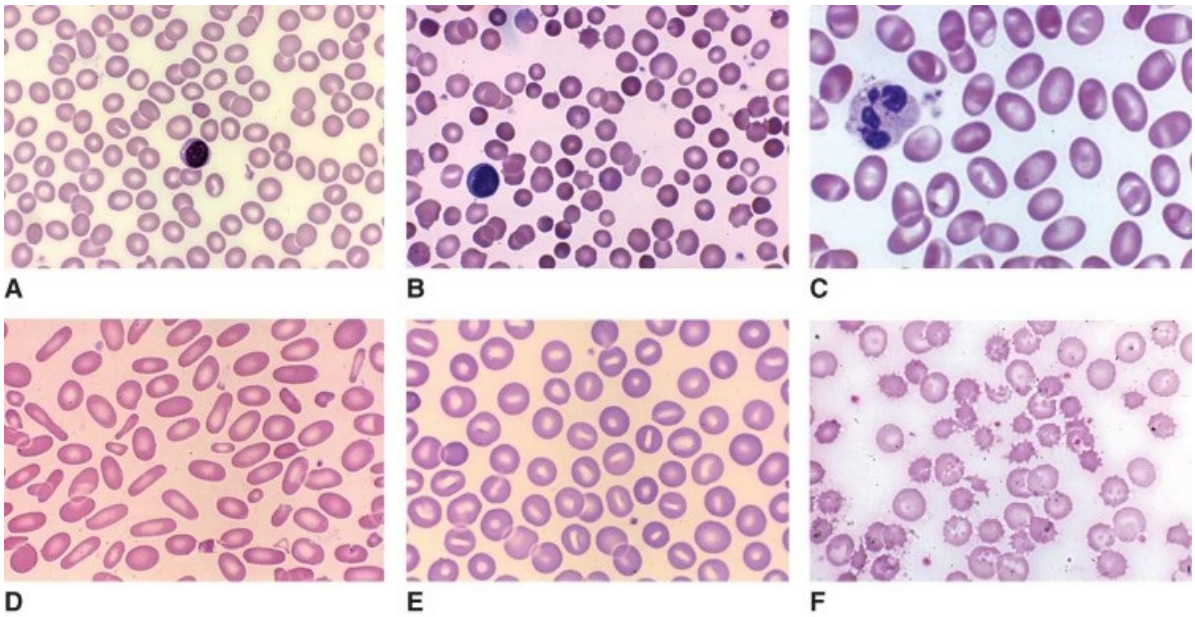


FIGURE 13–1 Blood films from patients with erythrocyte membrane disorders. **A.** Normal blood film. **B.** HS with dense spherocytes. **C.** SAO with large ovalocytes exhibiting a transverse ridge. **D.** HE with elongated elliptocytes and some poikilocytes. **E.** HSt with cup-shaped stomatocytes. **F.** Hereditary abetalipoproteinemia with acanthocytes. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

Table 13–1 summarizes the relationship between red cell membrane proteins and disease phenotype.

TABLE 13–1 ERYTHROCYTE MEMBRANE PROTEIN DEFECTS IN INHERITED DISORDERS OF RED CELL SHAPE

Protein	Disorder	Comment
Ankyrin	HS	Most common cause of typical dominant HS
Band 3	HS, SAO, NIHF, HAc	"Pincered" HS spherocytes seen on blood film presplenectomy; SAO results from 9 amino acid deletion
β-Spectrin	HS, HE, HPP, NIHF	"Acanthocytic" spherocytes seen on blood film presplenectomy; location of mutation in β-spectrin determines clinical phenotype
α-Spectrin	HS, HE, HPP, NIHF	Location of mutation in α-spectrin determines clinical phenotype; α-spectrin mutations most common cause of typical HE
Protein 4.2	HS	Primarily found in Japanese patients
Protein 4.1	HE	Found in certain European and Arab populations
GPC	HE	Concomitant protein 4.1 deficiency is basis of HE in GPC defects

GPC, glycophorin C; HAc, hereditary acanthocytosis; HE, hereditary elliptocytosis; HPP, hereditary pyropoikilocytosis; HS, hereditary spherocytosis; NIHF, nonimmune hydrops fetalis; SAO, Southeast Asian ovalocytosis.
Source: *Williams Hematology*, 9th ed, Chap. 46, Table 46–2.

HEREDITARY SPHEROCYTOSIS

Definition and Epidemiology

- Hereditary spherocytosis (HS) is characterized by osmotically fragile, spherical erythrocytes.
- HS occurs in all race groups but is the most common inherited hemolytic anemia in patients of

northern European descent.

Etiology and Pathogenesis

- HS is typically caused by a red cell membrane protein deficiency that compromises *vertical* interactions between the membrane skeleton and the lipid bilayer.
- Defects in spectrin, ankyrin, band 3, and protein 4.2 are common (see **Table 13–1**).
- The underlying molecular mutations are heterogeneous and may be family-specific.
- The membrane protein deficiency destabilizes the lipid bilayer, causing microvesicles to bud off from weakened areas, which leads to spherocyte formation.
- Spherocytes exhibit a decreased surface area-to-volume ratio and are dehydrated, which decreases their deformability.
- The passage of spherocytes through the spleen is impeded, and during erythrostasis they are engulfed by splenic macrophages and destroyed.

Inheritance

- HS is typically inherited in an autosomal dominant fashion.
- In approximately 25% of cases, HS is due to autosomal recessive inheritance or de novo mutations.
- Recessive HS is often caused by mutations in α spectrin or protein 4.2.

Clinical Features

- The typical clinical picture of HS combines evidence of hemolysis with spherocytosis and positive family history.
- The clinical manifestations of HS vary widely. Mild, moderate, and severe forms of HS have been defined according to differences in blood hemoglobin, bilirubin, and reticulocyte counts, which can be correlated with the degree of compensation for hemolysis as shown in **Table 13–2**.
- Severe cases may be diagnosed in infancy or childhood, but mild cases may escape detection until adulthood or may remain undetected.
- The majority of HS patients (60%–70%) have moderate disease with a variable degree of hemolytic anemia.
- Approximately 20% to 30% of HS patients have mild disease with compensated hemolysis where red blood cell production and destruction are balanced.
- As many as 10% of HS patients have severe disease in infancy. A small number of these, typically with autosomal recessive HS, present with life-threatening, transfusion-dependent anemia.
- An asymptomatic carrier state has been suggested in the case of clinically asymptomatic parents whose children present with typical HS.
- In the majority of HS cases, the clinical findings are limited to the erythroid lineage. However, a few kindred have cosegregating nonerythroid manifestations, particularly neuromuscular abnormalities and inherited distal renal tubular acidosis.

TABLE 13–2 **CLASSIFICATION OF HEREDITARY SPHEROCYTOSIS**

Laboratory Findings	HS Trait or Carrier	Mild Spherocytosis	Moderate Spherocytosis	Moderately Severe Spherocytosis*	Severe Spherocytosis†
Hemoglobin (g/dL)	Normal	11–15	8–12	6–8	< 6
Reticulocytes (%)	1–2	3–8	± 8	≥10	≥10
Bilirubin (mg/dL)	0–1	1–2	± 2	2–3	≥3
Spectrin content (% of normal)‡	100	80–100	50–80	40–80§	20–50
Blood film	Normal	Mild spherocytosis	Spherocytosis	Spherocytosis	Spherocytosis and poikilocytosis
Osmotic fragility					
Fresh blood	Normal	Normal or slightly increased	Distinctly increased	Distinctly increased	Distinctly increased
Incubated blood	Slightly increased	Distinctly increased	Distinctly increased	Distinctly increased	Markedly increased

*Values in untransfused patients.

†By definition, patients with severe spherocytosis are transfusion dependent. Values were obtained immediately prior to transfusion.

‡Normal, $245 \pm 27 \times 10^3$ spectrin dimers per erythrocyte.

§Spectrin content is variable in this group of patients, presumably reflecting heterogeneity of the underlying pathophysiology.

Reproduced with permission from Eber SW, Armbrust R, Schroter W: Variable clinical severity of hereditary spherocytosis: Relation to erythrocytic spectrin concentration, osmotic fragility, and autohemolysis. *J Pediatr* 1990;Sep;117(3):409-416.

Complications

- Chronic hemolysis leads to the development of bilirubin gallstones in approximately 50% of patients.
- Hemolytic crises are usually associated with viral illnesses and typically occur in childhood.
- Parvovirus B19 infection can precipitate an aplastic crisis with coexistent reticulocytopenia.
- Megaloblastic crises may occur in patients with increased folate demands such as during pregnancy.
- Lower leg ulcers or dermatitis develop in some patients but tend to heal quickly after splenectomy.
- In severe cases, extramedullary hematopoiesis from masses of erythroblasts simulating a tumor is seen.
- Severely affected individuals may develop iron overload, often but not entirely, due to frequent transfusions (see Chap. 9).

Laboratory Features

- Spherocytes on the blood film are the hallmark of the disease and are characterized by a smaller diameter, darker staining, and a decreased or absent central pallor, compared to normal red cells as depicted in **Figure 13–1B**.
- HS erythrocyte morphology is not uniform and ranges from very few spherocytes to large numbers of dense microspherocytes and in some cases poikilocytosis.
- “Pincered” red cells are often seen in band 3–deficient individuals, whereas spherocytic acanthocytes are associated with β-spectrin mutations.

- Erythrocyte indices (**Table 13–2**) reflect a mild to moderate decrease in hemoglobin in most patients and an increased mean (red) cell hemoglobin concentration (MCHC) in approximately 50% of cases.
- Markers of hemolysis include increased serum lactate dehydrogenase and unconjugated bilirubin, decreased haptoglobin concentration, and increased urobilinogen in the urine.
- HS red cells are osmotically fragile, and this has been exploited in various laboratory tests, including the glycerol lysis test and the cryohemolysis test. The standard osmotic fragility test measures the premature lysis of HS red cells in hypotonic salt solutions. Incubation of cells for 24 hours prior to measuring osmotic fragility improves sensitivity of the test (**Figure 13–2**). However, the disadvantage of this test that not all cases are detected and that it does not distinguish HS from other conditions with secondary spherocytes.
- Eosin 5′-maleimide is a fluorescent dye that binds to erythrocyte transmembrane proteins, and HS patients exhibit decreased fluorescence, although the sensitivity and specificity of the test vary, depending on the cutoff value.
- Biochemical and molecular diagnostics involve an initial analysis of the red cell membrane proteins by quantitative sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to identify the underlying defective protein. A simple DNA test as a part of the workup to diagnose HS is not feasible because HS is caused by mutations in several different genes and there are very few common mutations.

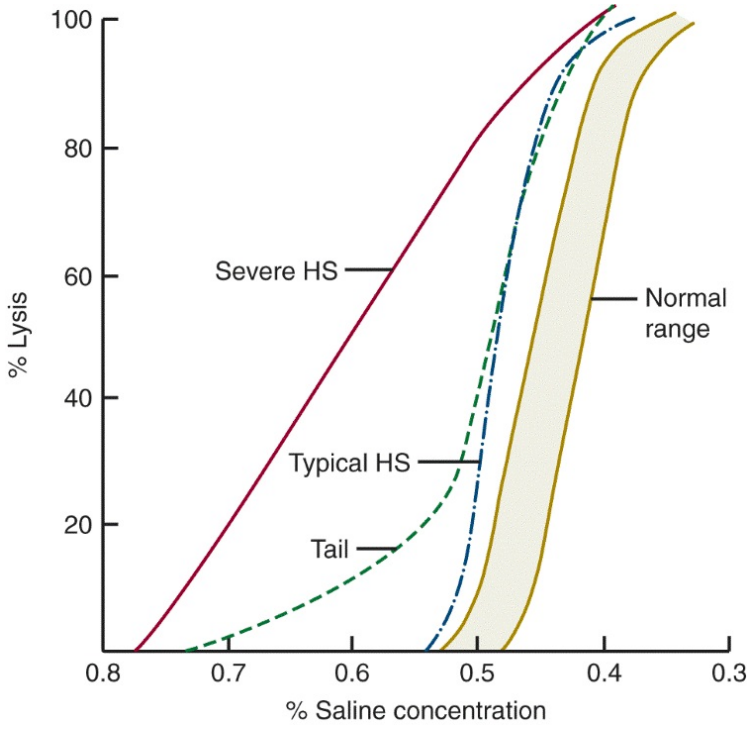


FIGURE 13–2 Osmotic fragility testing. The *shaded area* is the normal range. Results representative of both typical and severe spherocytosis are shown. A "tail," representing very fragile erythrocytes that have been conditioned by the spleen, is common in many HS patients prior to splenectomy. Reproduced with permission from Nathan DG, Orkin SH: *Nathan and Oski's Hematology of Infancy and Childhood*, 6th edition. Philadelphia; WB Saunders; 2003.

Differential Diagnosis

- Clinical features and family history should accompany an initial laboratory investigation comprising a complete blood count with a blood film, reticulocyte count, and serum bilirubin.

Children, parents, and siblings of the proband should have blood counts, a reticulocyte count, and blood film examined to identify affected family members.
- HS should be considered in patients with incidentally noted splenomegaly, gallstones at a young age, and parvovirus infections.
- Other causes of spherocytic hemolytic anemia should be excluded, particularly autoimmune hemolytic disease, by performing a direct antiglobulin test (Coombs test).
- HS may be obscured in disorders that increase the surface-to-volume ratio of erythrocytes, such as obstructive jaundice.

Therapy and Prognosis

- Patients with aplastic crises or severe hemolysis may require transfusion.
- Splenectomy cures or alleviates the anemia in the overwhelming majority of patients because splenic sequestration is the primary determinant of erythrocyte survival in HS.
- Patients with severe disease are good candidates for splenectomy, but in other cases the risk of overwhelming postsplenectomy infection, especially the emergence of penicillin-resistant pneumococci, has to be taken into consideration and weighed against the benefits.
- Splenectomy should be delayed until age 5 to 9 years, if possible, because of increased susceptibility to infection in younger children.
- Laparoscopic splenectomy has become the method of choice in centers with surgeons experienced in the technique.
- Occasionally, splenectomy may not correct the anemia, usually due to an accessory spleen.

HEREDITARY ELLIPTOCYTOSIS

Definition and Epidemiology

- Hereditary elliptocytosis (HE) is a heterogeneous disease characterized by the presence of elliptical or oval erythrocytes on the blood film (**Figure 13–1D**).
- Children, parents, and siblings of the proband should have blood counts, a reticulocyte count, and blood film examined to identify affected family members.
- HE occurs in all race groups but is more prevalent in individuals of African descent, possibly because elliptocytes may confer some resistance to malaria.

Etiology and Pathogenesis

- The primary abnormality in HE erythrocytes is defective *horizontal* interactions between protein components of the membrane skeleton that compromise its ability to maintain the biconcave disk-shape of the red cell during circulatory shear stress.
- Spectrin defects that impair self-association into tetramers and a deficiency of protein 4.1 are the most common underlying causes of HE (see **Table 13–1**).
- The molecular mutations are heterogeneous and but family-specific.
- In severe HE, red cell fragmentation may occur.

Inheritance

- HE is typically inherited as an autosomal dominant disorder, and de novo mutations are rare.

Clinical Features

- The clinical presentation of HE is heterogeneous, ranging from asymptomatic carriers to patients with severe, life-threatening anemia.
- The majority of HE patients are asymptomatic.
- Occasionally, severe forms of HE requiring red cell transfusion may present in the neonatal period, but hemolysis abates by 12 months of age, and the patient progresses to HE with mild anemia.

Laboratory Features

- The hallmark of HE is the presence of normochromic, normocytic elliptocytes on blood films as depicted in **Figure 13–1D**.
- Poikilocytes may be present in severe HE.
- The degree of hemolysis does not correlate with the number of elliptocytes.
- The reticulocyte count generally is less than 5% but may be higher when hemolysis is severe.
- Nonspecific markers of increased erythrocyte production and destruction are present.
- Specialized biochemical and molecular diagnostic tests involve analysis of the red cell membrane proteins by quantitative SDS-PAGE, as well as spectrin analysis to evaluate the spectrin dimer-to-tetramer ratio and to identify the abnormal spectrin domain. The defective gene may then be analyzed to elucidate the mutation.

Differential Diagnosis

- Acquired elliptocytes are associated with several disorders, including megaloblastic anemias, hypochromic microcytic anemias (iron-deficiency anemia and thalassemia), myelodysplastic syndromes, and myelofibrosis.
- Family history and the presence of other clinical features associated with the above diseases usually clarify the diagnosis.
- Specialized biochemical and molecular testing may additionally be used to establish a diagnosis of HE.

Therapy and Prognosis

- Therapy is rarely needed in HE patients.
- In severe HE cases, occasional red blood cell transfusions may be required and splenectomy has been palliative.

HEREDITARY PYROPOIKILOCYTOSIS

- Hereditary pyropoikilocytosis (HPP) is part of the HE spectrum of disorders.
- It is a rare autosomal recessive disorder typically found in patients of African origin.
- HPP is characterized by severe hemolytic anemia with marked microspherocytes and micropoikilocytes, and very few elliptocytes on the blood film.
- The mean (red) cell volume (MCV) is very low, ranging between 50 and 70 fL.
- HPP patients are often transfusion-dependent, and splenectomy is beneficial because the spleen is the site of erythrocyte sequestration and destruction.

- The molecular defects in HPP patients are a combination of *horizontal* (severely impaired spectrin tetramer formation) and *vertical* (spectrin deficiency) abnormalities, with the latter causing microspherocytes and exacerbating the hemolytic anemia.

SOUTHEAST ASIAN OVALOCYTOSIS

- Southeast Asian ovalocytosis (SAO) is widespread in certain ethnic groups of Southeast Asia.
- SAO is characterized by the presence of large, oval red cells, many of which contain one or two transverse ridges or a longitudinal slit (see **Figure 13–1C**).
- Typically, there is no clinical or laboratory evidence of hemolysis.
- SAO is a dominantly inherited disorder and homozygosity is postulated to be embryonic lethal.
- SAO erythrocytes are rigid and resistant to infection by several species of malaria parasites.
- SAO is caused by a nine–amino acid deletion in the hinge region of the band 3 protein.
- Rapid genetic diagnosis can be made by amplifying the defective region of the band 3 gene and demonstrating heterozygosity for the SAO allele containing the 27-bp deletion.

ACANTHOCYTOSIS

- Acanthocytes (spiculated red cells with multiple, irregular projections) and echinocytes (spiculated red cells with small uniform projections) occur in various inherited disorders and acquired conditions, as well as postsplenectomy.

Severe Liver Disease

- The anemia in patients with liver disease is often called “spur cell anemia.”
- Acanthocyte formation in spur cell anemia is a two-step process involving accumulation of free, nonesterified cholesterol in the red cell membrane and remodeling of abnormally shaped red cells by the spleen.
- Spur cell anemia is most common in patients with advanced alcoholic cirrhosis and characterized by rapidly progressive hemolytic anemia.
- Splenectomy is not advised because of severe liver disease.

Neuroacanthocytosis

- This is a heterogeneous group of rare disorders with variable clinical phenotypes and inheritance.
- The common features are a degeneration of neurons and abnormal acanthocytic erythrocyte morphology.
- These syndromes may be divided into: (1) lipoprotein abnormalities, which cause peripheral neuropathy, such as abetalipoproteinemia and hypobetalipoproteinemia; (2) neural degeneration of the basal ganglia resulting in movement disorders with normal lipoproteins, such as chorea-acanthocytosis and McLeod syndrome; and (3) movement abnormalities in which acanthocytes are occasionally seen, such as Huntington disease-like 2 and pantothenate kinase-associated neurodegeneration.

Abetalipoproteinemia

- This rare autosomal recessive condition is characterized by progressive ataxic neurologic disease.
- It is caused by a failure to synthesize or secrete lipoproteins containing products of the apolipoprotein B (apoB) gene.
- Patients exhibit mild anemia, and 50% to 90% of red cells are acanthocytic (**Figure 13–1F**).
- Steatorrhea develops early in life; retinitis pigmentosa and other progressive neurologic abnormalities lead to death in the second or third decade of life.
- Patients are treated with dietary restriction of triglycerides and supplementation with fat-soluble vitamins.

Chorea-Acanthocytosis Syndrome

- This rare autosomal recessive movement disorder is characterized by atrophy of the basal ganglia and progressive neurodegenerative disease with acanthocytosis.
- It is caused by an absence or markedly reduced levels of chorein, a protein involved in trafficking of membrane proteins.
- Lipoproteins are normal, and patients are not anemic.

McLeod Phenotype

- This is a rare X-linked defect of the Kell blood group system.
- It is caused by a deficiency of the XK protein, an integral membrane transporter component.
- Male hemizygotes who lack XK have up to 85% acanthocytes on the blood film with mild, compensated hemolysis and normal membrane lipids.
- Patients develop late-onset multisystem myopathy.
- Large deletions of the XK locus result in other coexisting disorders, such as Duchenne muscular dystrophy.

HEREDITARY STOMATOCYTOSIS SYNDROMES

- Stomatocytes are cup-shaped red cells characterized by a central hemoglobin-free area (**Figure 13–1E**).
- A net increase in cations causes water to enter the cells, resulting in overhydrated cells or stomatocytes, whereas a net loss of cations dehydrates the cells and forms xerocytes.
- Very rare conditions such as cryohydrocytosis show features intermediate between the two extreme phenotypes.
- Erythrocyte volume homeostasis is linked to monovalent cationic permeability, and this is disrupted in the hereditary stomatocytosis syndromes.
- These disorders of red cell cation permeability are very rare conditions that are inherited in an autosomal dominant fashion with marked clinical and biochemical heterogeneity.

Hereditary Stomatocytosis/Hydrocytosis

- This autosomal dominant disease is characterized by moderate to severe hemolytic anemia.
- It is caused by a marked passive sodium leak into the cell.

- Up to 50% stomatocytes are present and the osmotic fragility is increased.
- Red cell indices show decreased MCHC and a highly elevated MCV up to 150 fL.

Hereditary Xerocytosis (Desiccocytosis)

- This autosomal dominant disease is characterized by mild to moderate compensated hemolytic anemia.
- There is an efflux of potassium and red cell dehydration.
- The MCHC is increased, and red cells are resistant to osmotic lysis.

Other Stomatocytic Disorders

Rh Deficiency Syndrome

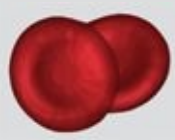
- The Rh complex is either absent or markedly reduced in patients with Rh deficiency syndrome.
- Patients present with mild to moderate hemolytic anemia.
- Stomatocytes and occasional spherocytes are seen on the blood film.
- Red cells have cation transport abnormalities, which cause dehydration.
- Splenectomy improves the anemia.

Familial Deficiency of High-Density Lipoproteins

- This rare condition with severe deficiency or absence of high-density lipoproteins leads to accumulation of cholesteryl esters in many tissues.
- Patients exhibit moderately severe hemolytic anemia with stomatocytosis.

Acquired Stomatocytosis

- Normal individuals have up to 3% stomatocytes on blood films.
- Acquired stomatocytosis is common in alcoholics and in patients with leukemias and lymphomas who have been treated with vinca alkaloids.



For a more detailed discussion, see Theresa L Coetzer: Erythrocyte Membrane Disorders, Chap. 46 in *Williams Hematology*, 9th ed.

CHAPTER 14
Hemolytic Anemia Related to Red Cell Enzyme Defects

Clinical manifestations of inherited red cell enzyme deficiencies are diverse and may entail:

- Hemolysis
  - — Acute and or episodic hemolysis after exposure to oxidants or infection, or after eating fava beans (favism)
  - — Chronic hemolytic anemia (hereditary nonspherocytic anemia)
- Icterus neonatorum
- Methemoglobinemia
  - — Chronic benign cyanosis
  - — Developmental defects with early fatality and chronic cyanosis
- Polycythemia
- No hematologic manifestations

However, only hemolytic complications will be reviewed here. Methemoglobinemia is reviewed in Chap. 18 and polycythemia in Chap. 27.

MECHANISM OF HEMOLYSIS IN PATIENTS WITH RED CELL ENZYME ABNORMALITIES

- In glucose-6-phosphate dehydrogenase (G6PD) deficiency, oxidant challenge leads to the formation of denatured hemoglobin (ie, Heinz bodies), which make the red cells less deformable and liable to splenic destruction.
- Metabolic aberrations in most red cell enzymopathies cause hemolysis by undefined mechanism(s).

GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

- G6PD is an X-linked disorder.
- The normal enzyme is designated G6PD B.
- A mutant enzyme with normal activity, or G6PD A(+), is polymorphic among persons of African descent. It has a single mutation at nt c. 376 (c. 376 A>G, amino acid substitution: p.Asn126Asp).
- G6PD A– is the principal deficient variant found among people of African ancestry. It has the nt c. 376 mutation and an additional mutation, almost always c. 202 G>A, p.Val68Met. G6PD A– has decreased stability in vivo, and affected hemizygotes have 5% to 15% of normal activity. Prevalence of G6PD A– in American men of African descent is 11%.

- G6PD deficiency in Europe is most common in the southern part of the continent and is most often a result of a Mediterranean variant that has a single base substitution at nt c. 563 (c. 563 C>T, p.Ser188Phe). Although there is scarcely any detectable enzymatic activity in the erythrocytes, there are no clinical manifestations unless the patient is exposed to oxidative drugs, infection, or fava beans. Other variants, such as G6PD Seattle (p.Asp282His) and G6PD A–, are also encountered in Europe.
- Many different G6PD mutations are also found in the Indian subcontinent and Southeast Asia. Most of these are severe variants. Examples include G6PD Canton, Viangchan, Bangkok, and Kaiping.

Drugs that Can Incite Hemolysis (see Table 14–1)

TABLE 14–1 DRUGS THAT CAN TRIGGER HEMOLYSIS IN GLUCOSE-6-PHOSPHATE DEHYDROGENASE–DEFICIENT INDIVIDUALS

Category of Drug	Predictable Hemolysis	Possible Hemolysis
Antiparasitics	Dapsone Primaquine Methylene blue	Chloroquine Quinine
Analgesics/Antipyretic	Phenazopyridine	Aspirin (high doses) Paracetamol (Acetaminophen)
Antibacterials	Cotrimoxazole Sulfadiazine Quinolones (including nalidixic acid, ciprofloxacin, ofloxacin) Nitrofurantoin	Sulfasalazine
Other	Rasburicase Toluidine blue	Chloramphenicol Isoniazid Ascorbic acid Glibenclamide Vitamin K Isosorbide dinitrate

Reproduced with permission from Luzzatto L, Seneca E: G6PD deficiency: A classic example of pharmacogenetics with on-going clinical implications, *Br J Haematol* 2014 Feb;164(4):469-480

- Individual differences in the metabolism of certain drugs as well as the specific G6PD mutation influence the extent of red blood cell destruction.
- Typically, drug-induced hemolysis begins 1 to 3 days after drug exposure. When severe, it may be associated with abdominal or back pain. The urine may become dark, even black.
- Heinz bodies appear in circulating red cells and then disappear as they are removed by the spleen. The hemoglobin concentration then decreases rapidly.
- Hemolysis is self-limited in the G6PD A– type but is the more severe and more prolonged in Mediterranean type and some Asian G6PD-deficient variants.

Febrile Illnesses that Can Incite Hemolysis

- Hemolysis may occur within 1 to 2 days of onset of a febrile illness, usually resulting in mild anemia.
- Hemolysis occurs especially in patients with pneumonia or typhoid fever.

- Jaundice may be particularly severe in association with infectious hepatitis.
- Reticulocytosis may be suppressed, and recovery from anemia is delayed until after the active infection is over.

Favism

- Favism is potentially one of the most severe hematologic consequences of G6PD deficiency.
- Hemolysis occurs within hours to days after ingestion of the beans.
- Urine becomes red or dark, and shock, sometimes fatal, may develop rapidly.
- Not all G6PD-deficient subjects develop hemolysis when they ingest fava beans. The enzyme deficiency is a necessary but not sufficient factor. The other factors required are not known, but believed to be, in part, genetic.
- More common in children than in adults, this condition is more likely with variants that cause severe deficiency.

HEREDITARY NONSPHEROCYTIC HEMOLYTIC ANEMIA (HNSHA)

- HNSHA may occur with severely deficient variants of G6PD deficiency (however, these are very rare; referred to as class 1 G6PD deficiency) and with deficiency of a variety of other red cell metabolic enzymes.
- Anemia may range from severe, transfusion-dependent, to a fully compensated state with near normal hemoglobin concentration.
- Chronic jaundice, splenomegaly, and gallstones are common, and some patients develop ankle ulcers.
- Nonhematologic manifestations may occur, such as neurologic abnormalities in glucose phosphate isomerase deficiency and phosphoglycerate kinase deficiency. Nonhematologic symptoms may sometimes even be predominant, such as myopathy in phosphofructokinase deficiency, or severe neuromuscular disease in triosephosphate isomerase deficiency. Even nontransfused subjects have increased risk of development of iron overload (see Chap. 9).
- Pyruvate kinase (PK) deficiency:
  — PK deficiency is the most common cause of HNSHA.
  — It is estimated to occur at the rate of approximately 50 per 1,000,000 in persons of European descent.
  — It can be so severe that chronic transfusion therapy is required.
  — A partial response to splenectomy is usually observed. As young PK-deficient red cells are selectively sequestered by the spleen in PK deficiency, the postsplenectomy response is accompanied by a paradoxical increase in the number of reticulocytes.
- Glucose phosphate isomerase deficiency:
  — This deficiency is the second most common cause of HNSHA.
  — Anemia is usually relatively mild, but fetal hydrops has been observed several times with this enzyme deficiency.
  — Response to splenectomy is usually good.
- Triosephosphate isomerase deficiency:
  — This deficiency is the most devastating of the red cell enzyme defects.

— Adults with the disease are rare because most patients die of neuromuscular complications before the age of 6 years.
- Pyrimidine 5′-nucleotidase deficiency:
  — This deficiency is characterized basophilic stippling (see Chap. 1) and is, therefore, the only cause of HNSHA in which a provisional diagnosis is possible from morphological analysis.
  — Acquired deficiency of pyrimidine-5′-nucleotidase may result from lead poisoning (lead preferentially occupies the enzyme's active site).

Laboratory Features

- Erythrocytes with enzyme deficiencies have normal morphology in the absence of hemolysis, except as noted above, or have mild changes that are not distinctive or specific.
- Increased serum bilirubin concentration, decreased haptoglobin levels, and increased reticulocyte counts may be present when hemolysis occurs.
- Secondary thrombocytosis may be present.
- High transferrin saturation and increased ferritin may be present.
- Mild to moderate leukopenia and thrombocytopenia may occur in patients with splenomegaly.

Differential Diagnosis

- This depends on demonstration of deficient enzyme deficiency.
- Start with screening tests for G6PD and PK deficiency.
- Enzyme tests may require retesting more than 2 months after patient is fully recovered from hemolytic episode because some enzymes levels are higher in reticulocytes and young red cells. This is especially common during a hemolytic episode in G6PD A– patients because residual young red cells have normal levels of G6PD.
- Assays or screening tests for G6PD deficiency are most reliable in healthy affected (hemizygous) males and may be normal in females with G6PD deficiency. DNA analysis allows for reliable confirmation of G6PD deficiency in female carriers.
- Family history and signs of nonhematologic pathologies can be very helpful in establishing the diagnosis.
- Presence of basophilic stippling suggests pyrimidine 5′-nucleotidase deficiency, if there is no evidence for lead poisoning.
- DNA analysis is recommended to enable genetic counseling is contemplated.

Treatment

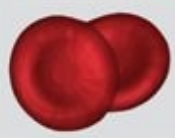
- G6PD-deficient individuals should avoid "oxidant" drugs (see **Table 14–1**).
- Transfusions should be given only in the most severe cases of G6PD deficiency, such as favism, but may be commonly required in PK or other enzyme deficiencies accompanied by severe anemia.
- Exchange transfusion may be necessary in infants with neonatal icterus when phototherapy fails (see Chap. 25).
- Splenectomy should be considered in certain patients with pyruvate kinase and triosephosphate isomerase deficiencies.

— Severity of disease and functional impairment are important considerations.
— If cholecystectomy is required, splenectomy may be done at the same time.

- If concomitant iron overload is present, iron chelation is indicated (see Chap. 9).
- Glucocorticoids are of no known value.
- Folic acid therapy is often given, but is without proven hematologic benefit unless a deficiency is found in the red cells.
- Iron therapy is contraindicated unless unrelated causes of iron deficiency are operative.
- An ongoing trial is testing an oral agent that stabilizes PK multimers and ameliorates PK metabolic defect.

ICTERUS NEONATORUM

- This condition may occur in some newborns with G6PD deficiency but also with other congenital enzyme and red cell membrane disorders (see Chap. 13). If not treated, it may lead to kernicterus and mental retardation.
- It is rare in neonates with the A– variant but more common in Mediterranean and various Asian variants.
- It occurs particularly in infants who are G6PD deficient or inheriting red cell membrane disorders who have also inherited a mutation of the UDP-glucuronosyltransferase-1 gene promoter (Gilbert syndrome).
- It results probably principally from in adequate bilirubin processing, but shortened red cell span plays a contributory role.



For a more detailed discussion, see Wouter W. van Solinge and Richard van Wijk: Disorders of Red Cells Resulting from Enzyme Abnormalities, Chap. 47 in *Williams Hematology*, 9th ed.

CHAPTER 15
The Thalassemias

DEFINITION

- Each of these disorders results from an inherited defect in the rate of synthesis of one or another of globin chains.
- Resultant imbalance of globin chain production may cause ineffective erythropoiesis, defective hemoglobin production, red cell hemoglobin precipitates, hemolysis, anemia of variable degree and propensity to iron overload.

ETIOLOGY AND PATHOGENESIS

Genetic Control and Synthesis of Hemoglobin

- Each hemoglobin (Hb) molecule consists of two separate pairs of identical globin chains.
- All normal human Hb molecules found in an adult have one pair of α-chains. The α-chains can combine with β-chains ($\alpha_2\beta_2$), δ-chains ($\alpha_2\delta_2$), and γ-chains ($\alpha_2\gamma_2$).
- Adult Hb is ~97% Hb A ($\alpha_2\beta_2$), ~0.5% Hb F α2γ2, and ~2.5% Hb A_2 ($\alpha_2\delta_2$).
- In fetal life, Hb F ($\alpha_2\gamma_2$) predominates. Position 136 of some γ-chains is occupied by glycine and in others by alanine. These are designated $^{G}\gamma$ and $^{A}\gamma$, respectively. At birth Hb F is a mixture of $\alpha_2{}^{G}\gamma_2$ and $\alpha_2{}^{A}\gamma_2$ in a ratio of 3:1.
- In embryonic life, Hb Gower 1 is composed of dimers of zeta (ζ) and epsilon (ε) globins ($\zeta_2\varepsilon_2$). Hb Gower 2 ($\alpha_2\varepsilon_2$) and Hb Portland ($\zeta_2\gamma_2$) are only present before the 8th week of intrauterine life.
- During fetal life, globin gene expression switches occur from ζ- to α- and from ∈- to γ-chain production, followed by β- and δ-chain production at perinatal life.

Globin Gene Clusters

- α-Gene cluster (chromosome 16) consists of one functional ζ gene and two α genes (α_2 and α_1).
- Exons of the two α-globin genes have identical coding sequences; however, they differ in second intron.
- Production of α_2 mRNA exceeds that of α_1, by factor of 1.5 to 3.
- β-Gene cluster (chromosome 11) consists of one functional ∈ gene, a $^{G}\gamma$ gene, an $^{A}\gamma$ gene, a pseudo β gene, a δ gene, and a β gene.
- Flanking regions contain conserved sequences essential for gene expression.

Regulation of Globin Gene Clusters

- Primary transcript is a large mRNA precursor, with both intron and exon sequences, which is extensively processed in the nucleus to yield the final mRNA.
- Expression of the globin genes is regulated by complex control mechanisms.

Developmental Changes in Globin Gene Expression

- β-Globin produced at low levels beginning at 8 to 10 weeks of fetal life increases considerably at about 36 weeks gestation.
- γ-Globin produced at high levels early starts to decline at ~36 weeks.
- At birth, β-globin and γ-globin production are approximately equal.
- By age 1 year, γ-globin production is less than 1% of total non–α-globin production.
- Mechanism of switches is being elucidated and involve BCL11A and leukemia-related factor encoded by the *ZBTB7A* gene.
- Fetal Hb synthesis may be reactivated in adults in some disease states by a yet unknown mechanism.

MOLECULAR BASIS OF THE THALASSEMIAS

- A large number of mutations cause thalassemia (eg, more than 200 for β-thalassemia).
- The molecular basis of the thalassemias is discussed in detail in Chap. 48 in *Williams Hematology*, 9th ed.

DIFFERENT FORMS OF THALASSEMIA (TABLE 15-1)

TABLE 15–1 THALASSEMIAS AND RELATED DISORDERS

α-Thalassemia
α^0
α^+
Deletion (–α)
Nondeletion (α^T)
β-Thalassemia
β^0
β^+
Normal hemoglobin A_2
Dominant
Unlinked to β-globin genes
δβ-Thalassemia
$(\delta\beta)^+$
$(\delta\beta)^0$
$(^A\gamma\ \delta\beta)^0$
γ-Thalassemia
δ-Thalassemia
δ^0
δ^+
εγδβ-Thalassemia

Hereditary Persistence of Fetal Hemoglobin
Deletion
$(\delta\beta)^0$, $(^A\gamma\ \delta\beta)^0$
Nondeletion
Linked to β-globin genes
$^G\gamma\ \beta^+$, $^A\gamma\ \beta^+$
Unlinked to β-globin genes

Source: *Williams Hematology*, 9th ed, Chap. 48, Table 48–1.

- β-Thalassemias are of two main varieties:
  — The two types are β^0-thalassemia, with total absence of β-chain production and β^+-thalassemia, with partial deficiency of β-chain production.
  — The hallmark of the common forms of β-thalassemias is elevation of Hb A_2 in heterozygotes.
- δβ-Thalassemias are heterogeneous:
  — In some cases, no δ- or β-chains are produced.
  — In other cases, the non-α chains are fusion δβ-chains: N-terminal residue of δ-chain fused to C-terminal residues of the β-chain. Fusion variants are called *Lepore hemoglobins*.
  — Levels of Hb F, but not HbA_2, are elevated in heterozygotes.
- Hereditary persistence of fetal Hb (HPFH):
  — HPFH is heterogeneous genetically (deletion and nondeletion forms).
  — It is characterized by persistence of Hb F in adult life.
  — Although it has no clinical significance, it may have mild thalassemic changes.
- α-Thalassemias are usually caused by deletion of one or more of the four α genes (two globin genes per haploid chromosome):
  — If one of the two α-globin loci on chromosome 16 is deleted, the designation α– is used. If both are deleted, the designation αα/– – is used. Thus, a patient with two α locus deletions would be designated α–/α– or αα/– – depending on the arrangement of the deletions on the chromosomes.
  — α-Thalassemias also arise from a variety of other mechanisms, such as an elongated α-chain because of a mutated stop codon (Hb Constant Spring) or missense or nonsense mutations.

PATHOPHYSIOLOGY

Imbalanced Globin Chain Synthesis (The Major Problem)

- Homozygous β-thalassemia (**Figure 15–1**):
  — β-Globin synthesis is absent or greatly reduced, resulting in hypochromic microcytic red cells.
  — Because excess α-chains are incapable of forming viable Hb tetramers, they precipitate in red cell precursors, resulting in intramedullary destruction of the abnormal erythroid cells (ineffective erythropoiesis) and hemolysis.
  — Clinical manifestations appear after neonatal switch from γ-chain to β-chain production.
- Heterozygous β-thalassemia:

— Usually only mild hypochromic microcytic anemia with elevated Hb A_2 is apparent.
— Some are more severe because of poor heme-binding properties and instability, with red cell inclusions containing precipitated β-chains as well as excess α-chains. These are sometimes designated as *hyperunstable hemoglobins*.

- α-Thalassemias:
  — There is defective α-chain production. Manifestations occur in both fetal and adult life because α-chains are present in both fetal and adult hemoglobin molecules.
  — In the newborn, excess γ-chains become soluble γ_4 homotetramers designated Hb Bart's.
  — After infancy, as the switch from γ- to β-chains takes place, excess β-chains if sufficiently large become β_4 homotetramer (Hb H).
  — Because both γ_4 and β_4 homotetramers are soluble, they do not precipitate to any significant degree, explaining the less severe degree of ineffective erythropoiesis in α-thalassemias compared to β-thalassemias.
  — However, Hb H is unstable and precipitates readily, forming inclusion bodies.
  — Both Hb Bart's and Hb H have high oxygen affinity.
  — Defect in Hb synthesis leads to hypochromic, microcytic cells.

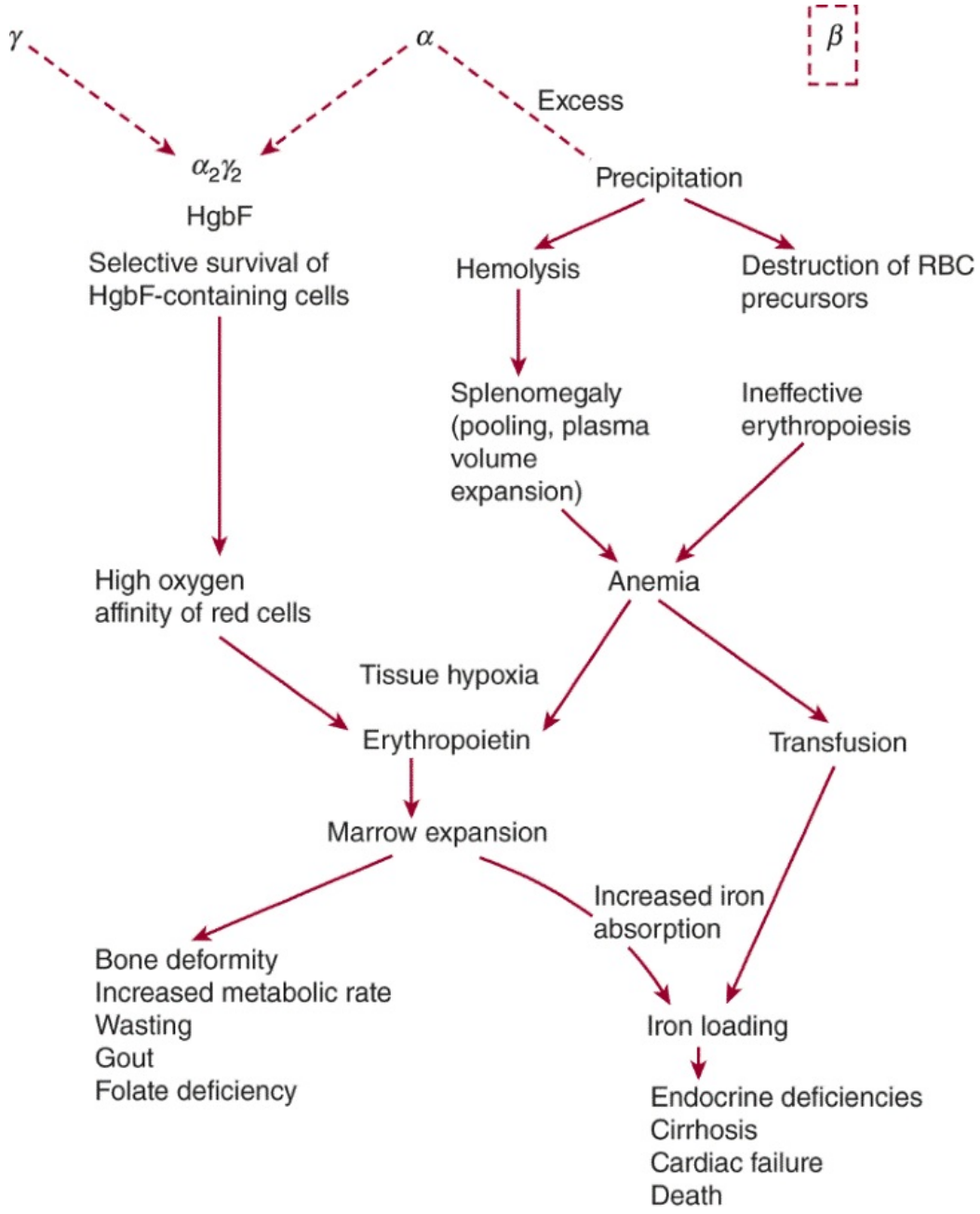


FIGURE 15–1 Pathophysiology of *β*-thalassemia. HgbF, hemoglobin F; RBC, red blood cell. (Source: *Williams Hematology*, 9th ed, Chap. 48, Fig. 48–13.)

Persistent Fetal Hemoglobin Production and Cellular Heterogeneity

- In β^0-thalassemias, Hb F is the only Hb produced except for small amounts of Hb A_2.
- In thalassemias, as in normal individuals, Hb F is heterogeneously distributed among the red cells.
- Because of elevated Hb F levels in β-thalassemias, red cells have high oxygen affinity.
- The mechanism of persistent γ-chain synthesis in thalassemias is incompletely understood.

Consequence of Compensatory Mechanisms for the Anemia of Thalassemia

- Severe anemia and the high oxygen affinity of Hb F in homozygous β-thalassemia produce severe tissue hypoxia.
- High oxygen affinity of Hb Bart's and Hb H accentuates hypoxia in severe forms of α-

thalassemia.

- Erythropoietin production and consequent expansion of marrow lead to deformities of skull with frequent sinus and ear infections, porous long bones, and pathologic fractures.
- Massive erythropoiesis diverts calories and also leads to hyperuricemia, gout, and folate deficiency.

Splenomegaly; Dilutional Anemia

- Constant exposure of the spleen to red cells of precipitated globin chains leads to "work hypertrophy" of spleen, ultimately leading to splenomegaly.
- The enlarged spleen may sequester red cells and expand plasma volume, exacerbating anemia.

Abnormal Iron Metabolism

- β^0-Thalassemia homozygotes accumulate large amounts of iron because of increased absorption and red cell transfusions.
- Iron accumulates in endocrine glands, liver and, most importantly, myocardium.
- Consequences are diabetes, hypoparathyroidism, hypogonadism, and death from heart failure.
- The role of hepcidin of erythroferrone in the abnormal regulation of iron absorption in thalassemias is discussed in Chap. 9 of this Manual.

Infection

- All forms of severe thalassemia appear to be associated with an increased susceptibility to bacterial infection; the iron overload may be one of the contributing factors. See Chap. 9 of this Manual.

Coagulation Defects

- Patients with thrombocytosis after splenectomy may develop progressive pulmonary hypertension with platelet aggregation in the pulmonary circulation.

Clinical Heterogeneity

- Most manifestations of β-thalassemia are related to excess α-chains.
- Degree of globin-chain imbalance determines severity.
- Coinheritance of α-thalassemia or of genes for enhanced γ-chain production may reduce the severity of β-thalassemias.

POPULATION GENETICS

- β-Thalassemias: Mediterranean populations, Middle East, India and Pakistan, Southeast Asia, southern Russia, China
  — Rare in Africa, except Liberia and parts of North Africa
  — Occurs sporadically in all races
- α-Thalassemias: widespread in Africa, Mediterranean populations, Middle East, Southeast Asia

— Loss of both functional α-globin loci on the same chromosome. This occurs in Mediterranean and Asian populations but is extremely rare in Africa and the Middle East. Thus, Hb Bart's hydrops syndrome and Hb H disease are largely restricted to Southeast Asia and Mediterranean populations.
- Thalassemic red cells: less likely to be infected with the plasmodial organisms of malaria

CLINICAL FEATURES

β-Thalassemias

- β-Thalassemia major: clinically severe, requiring transfusions
- β-Thalassemia intermedia: milder, later onset, requiring either few or no transfusions but at risk of iron overload
- β-Thalassemia minor: heterozygous carrier, clinically asymptomatic

β-Thalassemia Major

- Homozygous or compound heterozygous state
- Infant well at birth; anemia developing in first few months of life, becoming progressively more severe, and coinciding with switch from γ to β-chains; failure to thrive
- Onset of symptoms after first year of life more typical of β-thalassemia intermedia
- Inadequately transfused child
  — Stunted growth; expanded marrow leads to bossing of skull, expanded maxilla, widened diploë, gross skeletal deformities
  — Grossly enlarged liver and spleen; secondary thrombocytopenia and leukopenia
  — Skin pigmentation; chronic leg ulceration
  — Hypermetabolic state: fever, wasting, hyperuricemia
  — Frequent infections, folate deficiency, spontaneous fractures, dental problems
  — Symptoms of iron loading by time of puberty; poor growth; endocrine problems (diabetes mellitus, adrenal insufficiency); cardiac problems, death by the third decade as a result of cardiac siderosis
- Adequately transfused child
  — Grows and develops normally until effects of iron loading appear by end of first decade

β-Thalassemia Intermedia

- Wide spectrum of disability:
  — Severe forms: later-appearing anemia than β-thalassemia major; usually requires transfusion. There is retarded growth and development, skeletal deformities, and splenomegaly.
  — Milder forms: asymptomatic, transfusion-independent, Hb levels 10 to 12 g/dL.

β-Thalassemia Minor

- Often slight anemia without functional impairment; discovered by blood cell examination

α-Thalassemias

- Interactions of α-thalassemia haplotypes result in four broad phenotypic categories:
  — Normal (αα/αα).
  — Silent carrier (α–/αα).
  — α-Thalassemia trait (α–/α–) or αα/– –); mild hematologic changes, but no clinical abnormality; low mean cell volume (MCV) and low mean cell hemoglobin (MCH). There are varying levels of Hb Bart's (γ_4) at birth.
  — Hb H disease (– –/α–); hypochromic, severe to moderately severe hemolytic anemia often with marked splenomegaly. Red cells contain precipitates of Hb H(β_4), which is an unstable hemoglobin.
- Hb Bart's *hydrops fetalis* syndrome (– –/– –) is incompatible with extrauterine life.
  — Frequent cause of stillbirth in Southeast Asia. If alive at birth, infant dies within hours.
  — Pallor, massive edema, hepatosplenomegaly. Hydrops resembles that of Rh incompatibility.
  — High incidence of maternal toxemia of pregnancy, with enlarged placenta.
  — At autopsy: massive extramedullary hematopoiesis.

LABORATORY FEATURES

β-Thalassemias (Figure 15–2)

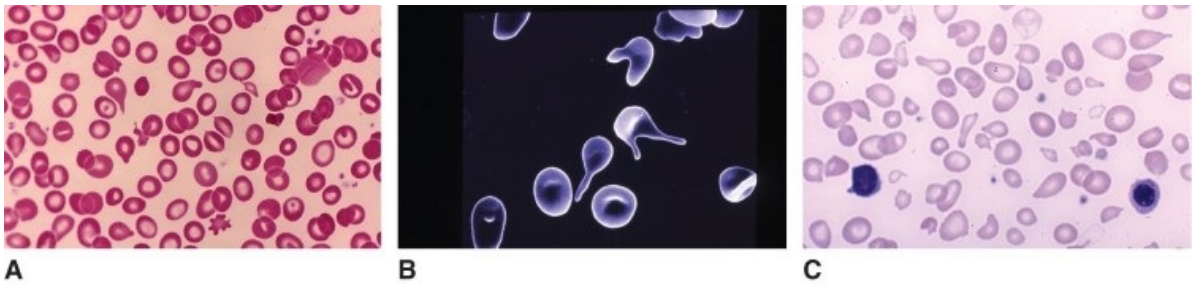


FIGURE 15–2 Blood films in *β*-thalassemia. **A.** *β*-Thalassemia minor. Anisocytosis, poikilocytosis, hypochromia. Occasional spherocytes and stomatocytes. **B.** Scanning electron micrograph of cells in **A** showing more detail of the poikilocytes. Note the knizocyte (pinch-bottle cell) at the lower right. **C.** *β*-Thalassemia major. Marked anisocytosis with many microcytes. Marked poikilocytosis. Anisochromia. Nucleated red cell on the right. Small lymphocyte on the left. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

β-Thalassemia Major

- Severe anemia: Hb 2 to 3 g/dL; blood film: marked anisopoikilocytosis, hypochromia, target cells, basophilic stippling, large poikilocytes; nucleated red cells numerous; reticulocytes moderately increased; inclusions of Hb in hypochromic red cells (these can be supravitally stained by methyl violet)
- After splenectomy: more inclusions; large, flat macrocytes; small, deformed microcytes
- Leukocyte and platelet counts normal or slightly elevated
- Marrow: marked erythroid hyperplasia, abnormal erythroblasts with stippling, increased sideroblasts; markedly increased storage iron
- Markedly ineffective erythropoiesis; shortened red cell survival
- Hb: Hb F increased, from less than 10% to greater than 90%; Hb A absent in β^0-thalassemia. Hb A_2 levels are low, normal, or high; always invariably elevated, however, if expressed as a proportion of Hb A

β-Thalassemia Minor

- Mild anemia: Hb 9 to 11 g/dL
- Microcytic hypochromic red cells: MCV 50 to 70 fL (MCV a valuable screen for thalassemia trait); MCH 20 to 22 pg
- Hb A_2 level: increased to 3.5% to 7%

α-Thalassemias

Hemoglobin Bart's Hydrops Fetalis Syndrome

- Blood film: severe thalassemic changes; many nucleated red blood cells
- Hb: Hb Bart's predominates; Hb Portland ($\zeta_2\gamma_2$) 10% to 20%

Hemoglobin H Disease

- Blood film: hypochromic microcytic red blood cells, increased polychromasia
- Mild reticulocytosis (~5%)
- Hb H inclusions demonstrable in almost all red blood cells in blood incubated with brilliant cresyl blue

α^0-Thalassemia Trait (αα/– – or α–/α–)

- Similar appearance of blood film and cell counts as in β-thalassemia trait
- 5% to 15% Hb Bart's at birth, disappears during maturation
- Rare cells with Hb H inclusions can be demonstrated in some cases

Silent Carrier or α^+-Thalassemia Trait (αα/α–)

- 1% to 2% Hb Bart's at birth in some but not all cases
- Gene mapping analysis is only certain method of diagnosing α-thalassemia carrier states

DIFFERENTIAL DIAGNOSIS

- For an approach to the diagnosis of thalassemia syndromes, see **Figure 15–3**.
- In childhood, hereditary sideroblastic anemias may resemble thalassemia, but marrow examination should permit differentiation (see Chap. 11).
- High fetal Hb levels found in juvenile chronic myelomonocytic leukemia rarely can cause confusion, but examination of the marrow should be definitive (see Chap. 46).
- Diagnosis of the rarer forms of thalassemia is discussed in Chap. 48, of *Williams Hematology*, 9th ed.

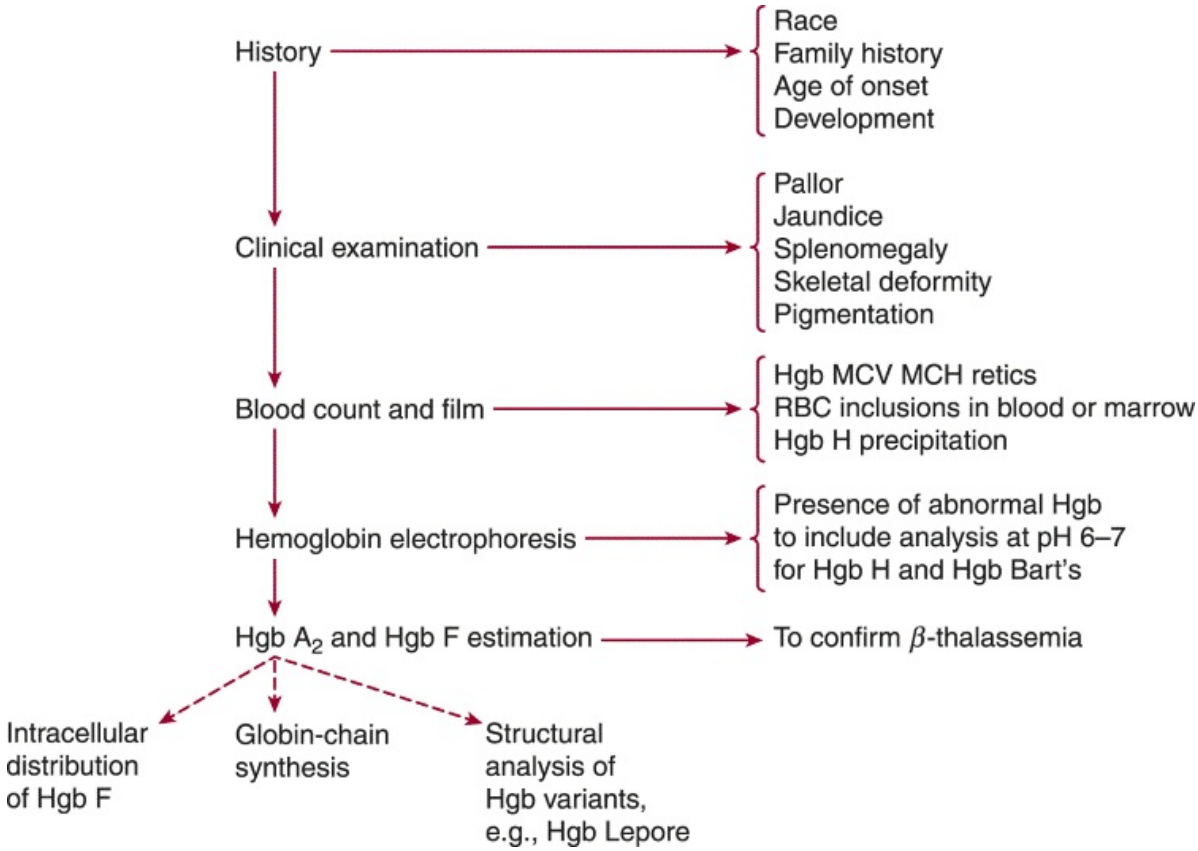


FIGURE 15–3 A flowchart showing an approach to the diagnosis of the thalassemia syndromes. MCH, mean cell hemoglobin; MCV, mean corpuscular volume; RBC, red blood cell count. (Source: *Williams Hematology*, 9th ed, Chap. 48, Fig. 48–20.)

THERAPY, COURSE, AND PROGNOSIS

β-Thalassemia Major

General Considerations

- High standard of pediatric care is required with adequate transfusions.
- Early treatment of infections is necessary.
- Folate supplements are warranted.
- Careful attention to respiratory infections and dental care must be taken because of bony deformities of skull.
- Preventive measures of iron overload are essential.
- When iron-loading is present, endocrine replacement therapy may be needed.

Allogeneic Hematopoietic Stem Cell Transplantation

- Very good results are obtained with human leukocyte antigen (HLA)–identical sibling donors if performed early.
- In the absence of risk factors (irregular chelation, hepatomegaly, portal fibrosis), approximately 90% of children have 5-year, event-free survival with a mortality risk of 4%.
- For patients with one or two risk factors, the disease-free survival rate is 82%; with all three risk factors, the disease-free survival rate is 51%.
- No case of hematologic malignancy has been observed after transplantation.

Transfusion

- In children, maintain Hb at 9.5 to 14 g/dL by transfusing red cells every 4 weeks to ensure normal growth and development. Use washed, filtered, or frozen cells to avoid transfusion reactions. Children maintained at high Hb level do not develop hypersplenism.

Iron Chelation

- Rationale: Every child on high-transfusion regimen will develop and die of myocardial siderosis.
- Subcutaneous infusion of deferoxamine, 12 hours, overnight: determine dose to achieve adequate urinary iron excretion.
- Continue nightly infusions of deferoxamine on outpatient basis and monitor by measurements of urinary iron excretion. Ascorbic acid administered orally, 50 to 100 mg/d, increases iron excretion but should be given only after deferoxamine infusion has been started.

Experimental Approaches

- Increase γ-globin synthesis in patients with β-thalassemia or sickle cell anemia using demethylating or cytotoxic agents, or arginine butyrate.
- Somatic gene therapy is discussed in Chap. 29 in *Williams Hematology*, 9th ed.

α-Thalassemia

Hydrops Fetalis (– –/– –)

- There is no treatment.
- Genetic counseling and prenatal diagnosis are encouraged.

Hb H Disease (α –/– –)

- Avoid "oxidant" drugs.
- Splenectomy may be needed if anemia and splenomegaly are severe.

PREVENTION

- For prenatal diagnosis, screen mothers at first prenatal visit; if mother is a thalassemia carrier, screen father. If both are carriers of gene for severe form of thalassemia, offer prenatal diagnostic testing and termination of pregnancy.
- Chorionic villus sampling at 9 to 10 weeks and fetal DNA analysis.

PROGNOSIS

β-Thalassemia

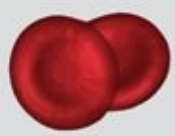
Thalassemia Major

- Cardiac complications have decreased dramatically in patients who are adequately treated by transfusion and iron chelation.
- Hematopoietic stem cell transplantation done early in life with HLA–identical sibling donors

can lead to cure.

Thalassemia Intermedia

- Patients may develop iron loading and severe bone disease in 3 to 4 decades.
- There is a high incidence of diabetes mellitus due to iron loading of pancreas.



For a more detailed discussion, see Stan Gerson: Gene Transfer Therapy, Chap. 29; Tomas Ganz: Iron Deficiency and Overload, Chap. 43; Sir David Weatherall: The Thalassemias, Chap. 48 in *Williams Hematology*, 9th ed.

CHAPTER 16

The Sickle Cell Diseases and Related Disorders

DEFINITIONS

- The molecular biology of hemoglobinopathies is well understood, but clinical progress in treatment has been limited. The vast majority of hemoglobinopathies are the result of single-nucleotide substitutions in the α, β, δ, or γ chains within the hemoglobin (Hb) tetramer.
- Hb variants are designated by letters of the alphabet, but after the letters of the alphabet were exhausted, newly identified variants were named according to the place in which they were first found (eg, Hb_{Zurich}). If they had a particular feature previously described by a letter, the location was added as a subscript (eg, Hb $M_{Saskatoon}$).
- In a fully characterized Hb variant, the amino acid position and change are described in a superscript to the appropriate globin chain (eg, Hb S, $\alpha_2 \beta_2^{6Glu\text{-}Val}$).

SICKLE CELL DISORDERS

- The term *sickle cell disorder* describes states in which sickling of red cells occurs on deoxygenation, not the genotype (ie, Hb SS, S/beta thal, SE).
- Hb S homozygosity (Hb SS), Hb SC, sickle cell–β thalassemia, and Hb SD produce significant morbidity and are therefore designated *sickle cell diseases*. These diseases are marked by periods of relative well-being interspersed with episodes of illness, but the severity of clinical manifestations varies widely among patients. Generally, sickle cell anemia is the most severe, but there is considerable overlap in clinical behavior among these diseases.

ETIOLOGY AND PATHOGENESIS

Hemoglobin Polymerization

- The Hb S mutation is the result of the substitution of valine for glutamic acid at position 6 in the β chain. Hb S polymerization is the central event in disease pathophysiology.
- Molecules of deoxyhemoglobin S have a strong tendency to aggregate and form polymers. Polymer formation alters the biophysical properties of the red cells, making them much less deformable and adherent to the endothelium.
- The sickling process is initially reversible, but repeated sickling and unsickling leads to irreversibly sickled cells because of membrane damage.
- Sickle cells lead to vascular stasis, tissue damage, and increase in microvascular blood viscosity.
- Susceptibility to sickling is dependent on several factors, including intracellular Hb

concentration (mean cell hemoglobin concentration, MCHC), presence of Hb other than Hb S that may interfere with the rate or degree of polymerization of Hb S (eg, Hb F), blood oxygen tension, pH, temperature, and 2,3-biphosphoglycerate levels.

- Some protection against sickling is conferred by elevated Hb F levels; apparently a threshold phenomenon exists, so that there is no effect beneath a certain level of Hb F.
- In the microvasculature, flow is affected by the rigidity of the sickled cells and adherence to the endothelium. Shear stresses in higher flow areas can break down the gel structure of Hb S. Because the duration of hypoxemia is also important, areas of vascular stasis (such as the spleen) with lower oxygen tension are particularly prone to vascular occlusion and infarction. Most patients with sickle cell anemia have splenic atrophy from multiple infarctions by early adulthood.

Other Pathways that are Key to the Pathophysiology of Sickle Cell Disease

- NO (nitric oxide) has vasodilatory, anti-inflammatory, and platelet aggregation effects. Chronic hemolysis releases free Hb into the circulation, which results in NO scavenging with consequent endothelial dysfunction and enhanced adhesion of sickled red cells.
- Several adhesion molecules and proinflammatory mediators (eg, tumor necrosis factor-alpha) are upregulated.
- Inflammatory stimuli lead to neutrophil, monocyte, and endothelial activation with increased white cell–red cell adhesion resulting in increased vaso-occlusion. Neutrophilia is an independent adverse prognostic factor in sickle cell anemia.
- Coagulation system is activated, and increased levels of tissue factor are present. Ischemia reperfusion injury with increased levels of reactive oxygen species occurs due to repeated vaso-occlusion followed by restoration of blood flow. Cation homeostasis is impaired because of red cell membrane injury, causing cellular dehydration and consequently increased intracellular Hb concentration. Some clinical findings such as isosthenuria in individuals with sickle cell trait are the result of this phenomenon. Abnormal adenosine signaling via the adenosine A2B receptor results in increased sickling, which may be key to clinical manifestations, such as priapism. Signaling through the adenosine A_{2A} receptor pathway of leukocytes and platelets results in an anti-inflammatory effect, which is being explored as a therapeutic strategy.

Inheritance

- Patients homozygous for the Hb S gene have inherited one gene from each parent. Because 8% of Americans of African ancestry have sickle trait, about 1 in 500 Americans of African descent are born with the Hb SS genotype.
- Occurrence of Hb SS disease can theoretically be prevented by detection of carriers and counseling regarding birth control or elective interruption of pregnancies with fetuses that are homozygous for Hb S.
- Although the sickle cell gene is found in a variety of areas (Middle East, Greece, India), its greatest prevalence is in tropical Africa, with heterozygote frequency as high as 40%. A geographic association with areas of high malaria prevalence has been determined to represent a lessened risk of developing falciparum malaria in heterozygotes. This enhanced

resistance to malaria is considered the reason for the persistent high prevalence of the mutation, given the lethality of the homozygous state in Africans.

CLINICAL MANIFESTATIONS

- The manifestations of all sickle cell diseases are sufficiently similar that they are discussed together here.
- High levels of Hb F protect against sickling for the first 8 to 10 weeks of life; thereafter, the manifestations of sickle cell disease may become apparent.
- There is great variability among affected individuals, but many patients are in good health most of the time.
- In children, most problems are related to pain, infection, or inflammation. In adults, clinical manifestations are likely to be more chronic, related to organ damage.

Crises

- *Vaso-occlusive* or *painful crises* are the most common manifestation. These occur with a frequency from almost daily to yearly; however, some affected individuals never have a painful crisis. Tissue hypoxia and infarction can occur anywhere in the body. It is important to carefully evaluate the patient to distinguish between painful crises and pain caused by another process.
- *Aplastic crises* occur when erythropoiesis is suppressed. Because red cell survival is greatly shortened in sickle cell disease, even temporary reduction in erythropoiesis is rapidly manifested by a dramatic fall in blood hemoglobin concentration. Infection (most notably parvovirus B19) usually causes this type of crisis, but it may also result from folic acid deficiency, which is of particular concern during pregnancy.
- *Sequestration crises* occur in infants and rarely in older children and adults with an enlarged spleen in sickle cell diseases, more likely in those other than Hb SS (eg, Hb SC). There is a sudden massive pooling of red cells in the spleen; this can cause hypotension and even death.
- *Hyperhemolytic episodes* occur uncommonly as a result of enhanced hemolysis in certain conditions, such as resolution phase of vaso-occlusive crisis where irreversibly sickled red cells are rapidly destroyed.

Other Clinical Manifestations

Cardiopulmonary System

- The “acute chest syndrome” consists of fever, leukocytosis, and a new pulmonary infiltrate. Infections or pulmonary fat microembolization are the two common causes of the acute chest syndrome. It is a leading cause of mortality from sickle cell disorders.
- Chronic pulmonary hypertension is another common manifestation in adult sickle cell disease patients. The likely reasons are NO scavenging, increased reactive oxygen species, increased arginase activity, and increased platelet activation. Pulmonary hypertension defined by right heart catheterization, elevated tricuspid regurgitant jet velocity of greater than or equal to 2.5 m/s and an elevated N-terminal of the prohormone brain natriuretic peptide (NT-proBNP)

level of greater than or equal to 160 pg/mL confer an increased mortality risk.
- Asthma, abnormal pulmonary function tests, and airway hyperreactivity are other pulmonary presentations.
- Tachycardia and high-output cardiac (flow) murmurs are commonly seen, especially during vaso-occlusive episodes.

Central Nervous System
- Thrombotic strokes occur more commonly in children, usually without warning. Risk is highest during first decade of life. Recurrence is common (in at least two thirds), usually within 3 years. Older adults may have increased risk of hemorrhagic stroke.
- The best predictor for thrombotic stroke risk is an increased blood flow velocity in major intracranial arteries on transcranial Doppler (TCD) ultrasound.
- Patients with two abnormal readings defined as TCD velocities greater than 200 cm/s should be offered a chronic red cell transfusion program for primary stroke prevention. A strategy of switching to hydroxyurea after a year of transfusion along with phlebotomy to reduce iron overload appears to be noninferior to indefinite transfusion for primary stroke prevention. Silent brain infarcts, defined as an abnormal T2 signal on magnetic resonance imaging (MRI), begin in infancy and progress during childhood. They may occur despite chronic transfusion therapy. Neurocognitive decline secondary to anemia and hypoxemia occur even in those with a normal brain MRI.

Genitourinary System
- The environment of the renal medulla (hyperosmolar, hypoxic) predisposes to sickling. Hyposthenuria, papillary necrosis, and hematuria are commonly present.
- Priapism is most commonly seen in Hb SS disease, whereas nocturnal enuresis is prevalent in approximately 30% of the adolescent sickle cell disease population.
- The prevalence of microalbuminuria and proteinuria increases with age. Infants with sickle cell disease have glomerular hyperfiltration. This may evolve into microalbuminuria, proteinuria, and to chronic kidney disease/end-stage renal disease.

Musculoskeletal System
- Young children with Hb SS tend to be short. Puberty is delayed, but growth occurs in late adolescence and adults are of normal size.
- Erythroid hyperplasia in the marrow results in widening of the medullary spaces and thinning of the cortex. The vertebral bodies may show biconcavities on the upper and lower surface (codfish spine).
- Bone infarctions can be followed by periosteal reaction and areas of osteosclerosis. Dactylitis occurs in children, usually up to 4 years of age, because hematopoietic marrow is still present in the bones of the hand and feet. In adults, avascular necrosis occurs chiefly in the femoral and humeral heads. About 50% of adults with sickle cell anemia will have femoral head avascular necrosis by age 33. Chronic transfusion and hydroxyurea do not impact incidence of avascular necrosis; joint replacement is needed in advanced cases. Osteopenia, osteoporosis and fractures of long bones are prevalent and likely under-reported.

Spleen

- In Hb SS disease, splenomegaly (but poor splenic function) in childhood is followed by repeated infarction, leaving a small fibrotic spleen in the adult (autosplenectomy). However, splenomegaly usually persists in patients with Hb SC, SE, or sickle β-thalassemia.

Hepatobiliary System

- About one third of sickle cell disease patients will manifest hepatic dysfunction of multifactorial origin.
- Sickle cell–induced cholestasis can be very serious and even fatal, although exchange transfusion has been reported as an effective treatment.
- Hepatitis may develop from transfusions, usually in regions in which testing for hepatitis B and C virus in blood is not performed fastidiously.
- The liver, sometimes chronically enlarged, can also enlarge transiently during a painful crisis (hepatic sequestration crisis).
- Gallstones are seen in 50% to 75% of adults; they have been seen in children as young as 6 years of age. Although there is some debate, patients with asymptomatic cholelithiasis probably should not be subjected to surgery.

Iron Overload

- Organ effects from iron overload are being recognized increasingly in adults with sickle cell disease; they develop in patients who have been transfused repeatedly (see Chap. 9).

Eye

- Neovascularization occurs after obstruction of retinal vessels, resulting in a proliferative retinopathy; however, spontaneous regression can occur in up to 60% of cases. Laser coagulation can prevent this complication. This is more common in Hb SC disease than in Hb SS disease.

Leg Ulcers

- Leg ulcers occur with varying frequency in adults and are related to multiple factors (low blood Hb concentration, brisk hemolysis, stasis). They typically occur on the lower extremity with the medial malleolus area more likely to be affected than the lateral malleolus.

Infection

- Children younger than 5 years of age are susceptible to infection by encapsulated organisms due to functional asplenia.

Pregnancy

- Complications to the mother include increased frequency of sickle cell painful crises, preeclampsia, and infections.
- Complications to the fetus include miscarriage, intrauterine growth restriction, preterm birth, low birth weight, and stillbirth and newborn death.
- Oral contraceptives may slightly increase the risk of thromboembolism, but this is less of a risk than pregnancy. Given insufficient data, contraception advice is similar to that of the non–

sickle cell disease population. Routine transfusion has not been shown to be beneficial; transfusion should be undertaken for Hg of less than 6 g/dL, given an increased risk of abnormal fetal oxygenation and fetal death reported in the non–sickle cell disease population.

Laboratory Features

- The Hg level is usually between 5 and 11 g/dL. Anemia is normochromic and normocytic, but considerable variation in red cell size and shape is noted. Sickled cells and target cells are seen on the blood film; reticulocytosis is almost always present (**Figure 16–1**).
- Leukocytosis and thrombocytosis are common, even in patients without acute problems; these may be caused by a reactive marrow along with demargination of peripheral leukocytes and by functional asplenia.
- Elevation in whole body iron burden is common; however, clinically significant hemochromatosis is rare.
- Hb electrophoresis is utilized to detect Hb S. Hb A_2 and often Hb F are particularly increased in patients with sickle cell–β thalassemia; however, many laboratories cannot accurately measure Hb A_2 in the presence of Hb S. Despite high levels of Hb F at birth, electrophoresis can detect Hb S in the newborn.
- Prenatal diagnosis is performed by examining DNA from a chorionic villus biopsy or from cells obtained at amniocentesis.

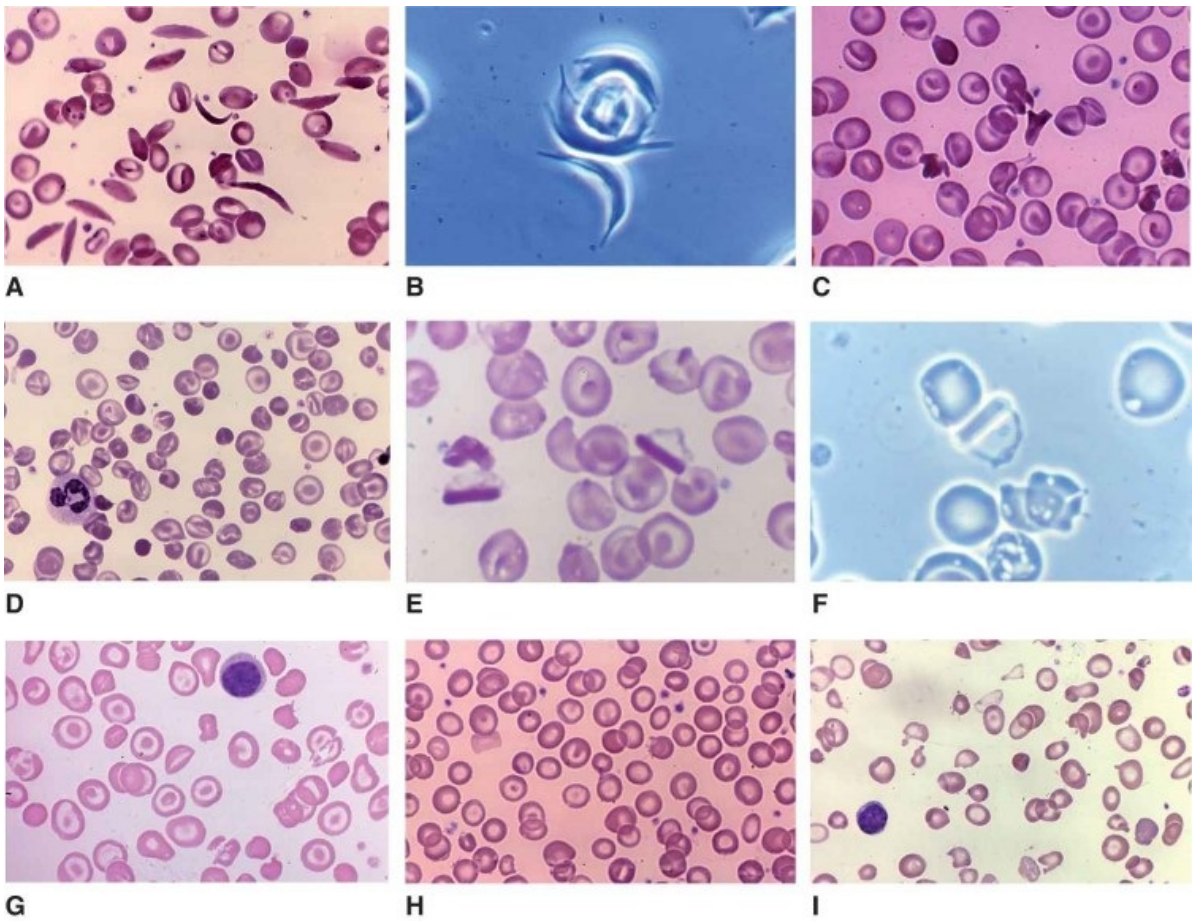


FIGURE 16–1 Blood cell morphology in patients with structural hemoglobinopathies. **A.** Blood film. Hemoglobin (Hb) SS disease with characteristic sickle-shaped cells and extreme elliptocytes with dense central hemoglobin staining. Both shapes are characteristic of sickled cells. Occasional target cells. **B.** Phase contrast microscopy of wet preparation. Note the three sickled cells with terminal fine-pointed projections as a result of tactoid formation and occasional target cells. **C.** Hb SC disease. Blood

film. Note high frequency of target cells characteristic of Hb C and the small dense, irregular, contracted cells reflective of their content of Hb S. In effect, these are atypically shaped sickle cells. **D.** Hb CC disease. Blood film. Characteristic combination of numerous target cells and a population of dense (hyperchromatic) microspherocytes. Of the nonspherocytic cells, virtually all are target cells. **E.** Hb CC disease postsplenectomy. Blood film. Note the rod-like inclusions in two cells as a result of Hb C paracrystallization. These cells are virtually all removed in patients with spleens. **F.** Hb CC disease postsplenectomy. Phase contrast microscopy of wet preparation. Note the Hb C crystalline rod in a cell. **G.** Hb DD disease. Blood film. Note frequent target cells admixed with population of small spherocytes, poikilocytes, and tiny red cell fragments. **H.** Hb EE disease. Blood film. Hypochromia, anisocytosis, and target cells. **I.** Hb E thalassemia. Blood film. Marked anisocytosis (primarily microcytes) and poikilocytosis. Hypochromia. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

TREATMENT

Nonspecific Measures

- The administration of folic acid may be useful. Pneumococcal vaccine should be given to children and to those adults who have not received it. Penicillin prophylaxis is administered up to the age of 5 years. Penicillin prophylaxis beyond age 5 may be considered in patients with a surgical splenectomy and those with recurrent pneumococcal infections. Infections should be identified and treated early.

Specific Measures

- Hydroxyurea
  - — Chronic administration at a starting dose of 15 mg/kg orally per day has been shown to decrease the incidence and severity of painful crises. Therefore, it should be considered for patients with frequent and severe crises or occurrence of other disease manifestations, including acute chest syndrome. Therapy is associated with improved survival, and this agent is underutilized in sickle cell disease patients. Therapy may be safely given to infants as young as 6 months of age. The dose may be escalated to 35 mg/kg with careful monitoring of blood counts; caution should be exercised in those with renal failure. The drug is held during pregnancy, although no teratogenic or leukemogenic effect has been observed to date. The precise mechanism of the hydroxyurea effect is uncertain. It was used initially to increase red cell Hb F, but this does not occur in most sickle cells in treated patients and is quantitatively modest. The drug decreases the neutrophil concentration in the blood. Neutrophils play a key role in fostering sickle cell crisis. Both effects may play a role. Other agents are being studied that can encourage the switch from β to γ Hb chains, resulting in higher Hb F in the cells.
- Allogeneic hematopoietic stem cell transplantation
  - — This is the only curative treatment for sickle cell disease, but because of the attendant risks, including death, it is suitable only for carefully selected patients with a human leukocyte antigen (HLA)–matched donor.
- Red cell transfusion
  - — This treatment is used frequently in sickle cell disease to increase hemoglobin concentration and to decrease the proportion of sickle cells in the blood. Chronic red cell transfusion therapy has been conclusively demonstrated to prevent strokes (see Chap. 91 for considerations of chronic red cell transfusion therapy).
  - — Transfusions for chronic asymptomatic anemia or routine vaso-occlusive episodes should be avoided.

— Typical indications include acute chest syndrome, stroke prevention or treatment, aplastic and sequestration crises, multiorgan failure, and transfusion prior to surgery.

Management of Complications

- Patients in vascular crises should be kept warm and given adequate hydration and pain control; oxygen is beneficial only for hypoxic patients. Overhydration should be avoided. The period of crisis usually resolves in hours to days. Hydroxyurea therapy (see "Specific Measures," above) may be considered for prevention or decreased frequency of recurrences.
- Patients undergoing anesthesia are at increased risk for a crisis and should be observed closely for development of hypoxia or acidosis, which could precipitate a crisis. Transfusion has been shown to decrease the risk of clinically significant complications.
- The acute chest syndrome is a life-threatening complication, and exchange transfusions or red cell transfusions appear beneficial along with adequate pain control, bronchodilators, antibiotics (include coverage of atypical organisms), and incentive spirometry.
- Because strokes in children are a recurring complication, vigorous therapy of children who have had this complication is recommended. A regular transfusion program is instituted to reduce Hb S levels below 30%. Hematopoietic stem cell transplantation can be considered for children with an HLA-matched sibling.
- Priapism, if recent, should be treated immediately by rapid hydration, red cell transfusion, and analgesia for a short period of observation, while awaiting an urgent urologic consultation. If unsuccessful, urologic intervention, usually by injection of a dilute solution of epinephrine into the corpus cavernosum, can be performed. This approach has a high frequency of success and preserves penile function. Surgical procedures, such as shunts, should be avoided if at all possible. Maintenance therapy with an oral α-adrenergic blocker, such as phenylephrine, can be used.
- Patients should be closely watched during pregnancy. Transfusion should follow general guidelines for sickle cell disease; prophylactic transfusions are undertaken for Hg of less than 6 g/dL.
- Bed rest, elevation, and zinc sulfate dressings are used to treat leg ulcers. A transfusion program or skin grafting can enhance healing. Often, they are quite resistant to therapeutic measures and require a long time to heal. Iron overload is managed by iron chelators: desferrioxamine given subcutaneously at a dose of 25 to 40 mg/kg per day or deferasirox given orally at a dose of 20 to 40 mg/kg per day. Auditory and ophthalmologic examinations should be performed at least annually while on iron chelation therapy; because deferasirox carries a risk of renal failure and hepatic damage, hepatic and renal function should be monitored closely.

SICKLE CELL TRAIT

- In sickle cell trait, less than half of the Hb in each red blood cell is Hb S (approximately 40%) and the rest is normal Hb, principally A. This effectively protects against sickling except under special circumstances, such as severe hypoxia or the hyperosmolarity encountered in the renal circulation.

- Numerous anecdotal reports suggest that sickle cell trait may be injurious, but the morbidity and mortality are extremely low and difficult to quantify.
- Individuals with sickle cell trait are at increased risk of sudden death and splenic infarction under severe environmental conditions. Hyposthenuria, renal papillary necrosis causing microscopic or gross hematuria, and rarely renal medullary carcinoma can be seen. There is an increased risk of venous thromboembolism as well.

HEMOGLOBIN C DISEASE

- Glutamic acid in the sixth position of the β chain is replaced by lysine in Hb C.
- In the homozygous state, most of the Hb in the cell is Hb C, the red blood cells are more rigid than normal, and intracellular crystals of Hb C are found; target cells are numerous. In addition, a population of spherocytes is a characteristic finding.
- In Americans of African descent, the prevalence of the heterozygous state (Hb C trait), which is asymptomatic, is approximately 2%.
- Splenomegaly and mild hemolytic anemia are almost always present in the homozygous state. Some patients develop bilirubin gallstones.
- No treatment is required, and the prognosis is excellent.

HEMOGLOBIN D DISEASE

- These Hb variants have normal solubility but migrate like Hb S on electrophoresis.
- The highest prevalence is in northwest India (2%–3%).
- The heterozygous state as well as the homozygotes are asymptomatic with normal red cell indices.
- Hemoglobin SD occurs rarely and presents as severe sickle cell disease. Hb D-β thalassemia is also rare.

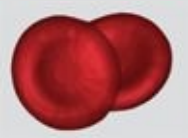
HEMOGLOBIN E DISEASE

- A β-chain mutation ($\beta^{26Glu\text{-}Lys}$) results in Hb designated Hb E. Some of the Hb E mRNA undergoes alternative splicing, giving rise to a thalassemia-like picture.
- This is a relatively common abnormal Hb in Southeast Asia.
- Hb E trait is asymptomatic, but mild microcytosis occurs.
- In association with β thalassemia, a moderate anemia and splenomegaly are present; splenectomy may be considered in this setting.
- Homozygous patients have been described; they have microcytosis and mild anemia.

OTHER HEMOGLOBINOPATHIES

- Many other abnormal Hg molecules have been described; most are uncommon and of no clinical significance. Others can produce cyanosis because of a low oxygen affinity,

erythrocytosis because of a high oxygen affinity, or a hemolytic anemia because of instability. These are described in Chaps. 17, 18, and 27.



For a more detailed discussion, see Kavita Natarajan, and Abdullah Kutlar: Disorders of Hemoglobin Structure: Sickle Cell Anemia and Related Abnormalities, Chap. 49 in *Williams Hematology*, 9th ed.

CHAPTER 17

Hemoglobinopathies Associated with Unstable Hemoglobin

DEFINITION

- The unstable hemoglobins discussed here result from a mutation that changes the amino acid sequence of one of the globin chains, leading to instability and precipitation of the hemoglobin molecule.
- Homotetramers of normal β chains (hemoglobin H) or, less often, γ chains (hemoglobin Bart's) are also unstable. These hemoglobins are found in α-thalassemia (see Chap. 15).

ETIOLOGY AND PATHOGENESIS

- The tetrameric hemoglobin molecule has numerous noncovalent interactions that maintain the structure of each subunit and bind the subunits to each other.
- Amino acid substitutions or indels that weaken noncovalent interactions allow hemoglobin to denature and precipitate as insoluble globins, which may attach to the cell membrane, forming Heinz bodies.
- Heinz bodies impair erythrocyte deformability, impeding the ability to negotiate the splenic sinuses; "pitting" of Heinz bodies causes loss of membrane area and ultimately destruction of red cells, reflected in a hemolytic anemia.

INHERITANCE

- These are autosomal dominant disorders. The patients are heterozygotes and have a combination of hemoglobin A and unstable hemoglobin in their red cells. Homozygous and compound heterozygotes are not observed; the conditions are thought to be lethal.
- Sometimes patients develop an unstable hemoglobin as a de novo mutation. More than 80% of patients have a defect in the β globin chain. Defects in the α globin chain are less likely to cause a clinical disorder because genome has four α-globin genes, and a mutation in one gene results in a minor proportion of abnormal globin in the cell.

CLINICAL FEATURES

- Decreased hemoglobin concentration is variable from virtually normal to severely decreased. Patients may have reticulocytosis, elevated indirect bilirubin, elevated lactic dehydrogenase, and decreased to absent haptoglobin. The blood film shows polychromasia, anisocytosis, poikilocytosis, and, sometimes, basophilic stippling (Heinz bodies).

- Hemolysis is usually compensated. A patient with an unstable hemoglobin with high oxygen affinity may have a hemoglobin level in the upper normal range.
- The treatment with oxidant drugs may precipitate hemolytic episodes, making the diagnosis apparent.
- In β-chain mutations, chronic hemolytic anemia becomes evident after neonatal period, because during the first year of life γ chains (fetal hemoglobin) are replaced by mutant β chains.
- Physical findings may include pallor, jaundice, and splenomegaly.
- Some patients have dark urine, probably from the catabolism of free heme groups or Heinz bodies.

LABORATORY FEATURES

- Hemoglobin concentration may be normal or decreased. The mean corpuscular hemoglobin may be decreased because of loss of hemoglobin from denaturation and pitting.
- Blood film may show hypochromia, poikilocytosis, polychromasia, anisocytosis, and basophilic stippling.
- Heinz bodies are commonly found in circulating red cells; after splenectomy they become more frequent.
- Reticulocytosis is often disproportionate to the severity of the anemia, particularly when the abnormal hemoglobin has a high oxygen affinity.
- Diagnosis of an unstable hemoglobin is confirmed by one of the following:
  — Isopropanol precipitation test: a simple screening test that involves the incubation of the hemolysate with a 17% solution of isopropanol. Hemolysates containing unstable hemoglobin variants form a precipitate, whereas a normal hemolysate remains clear. This is a very sensitive but not entirely specific test for unstable hemoglobins.
  — Heat denaturation test: more cumbersome, time-consuming, but as specific as isopropanol test. However, it is less available.
  — Heinz body detection: requires the incubation of erythrocytes with a supravital stain. However, Heinz bodies are not specific for unstable hemoglobins (**Figure 17–1**).
  — Hemoglobin electrophoresis: may be useful but insensitive because a normal pattern does not rule out an unstable hemoglobin. Thus, electrophoresis is not a screening or reliable test for unstable hemoglobins.
- Some unstable globin variants can be better identified by reverse phase high performance liquid chromatography, because of changes in their hydrophobicity but again with not perfect specificity.
- Determination of hemoglobin oxygen affinity ($P_{50}O_2$) is the correct test to detect unstable hemoglobins with altered oxygen-hemoglobin affinity.
- Specific mutation of unstable hemoglobins can only be identified by DNA analysis.

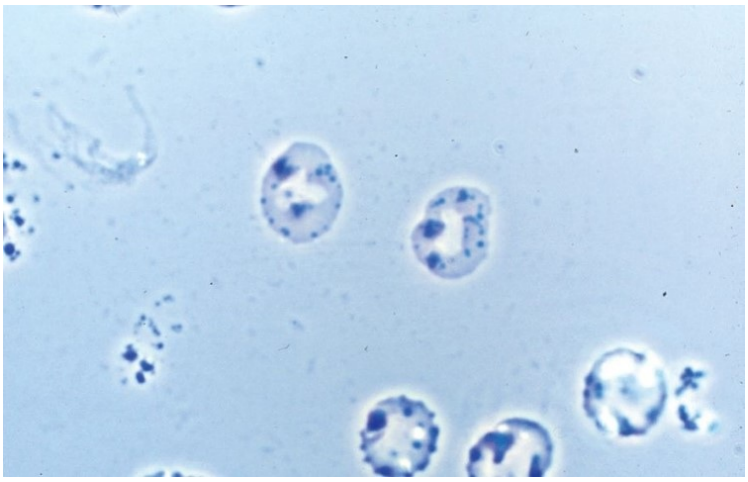


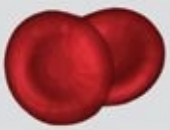
FIGURE 17–1 Wet preparation stained with crystal violet. Inclusions in red cells (Heinz bodies) are usually attached to membrane. (Reproduced with permission from *Lichtman's Atlas of Hematology,* www.accessmedicine.com.)

DIFFERENTIAL DIAGNOSIS

- Consider the possibility of an unstable hemoglobin in all patients with a hereditary nonspherocytic hemolytic anemia (see Chap. 14), especially with hypochromic red cells and reticulocytosis out of proportion to the degree of anemia.
- Not all patients with a positive test for unstable hemoglobin have this disorder; a false-positive isopropanol stability test may be seen in patients with sickle hemoglobin, elevated methemoglobin, or hemoglobin F.
- Hemoglobin H and hemoglobin Bart's are also unstable. These can be detected by electrophoresis and are found in patients with α-thalassemia.

TREATMENT, COURSE, AND PROGNOSIS

- Most patients have a relatively benign course.
- Gallstones are common, often requiring cholecystectomy.
- Hemolytic episodes may be precipitated by administration of oxidative drugs or less often during infections.
- Treatment is usually not required. Folic acid is often given, although benefit is not proven. Splenectomy may be useful in some patients but may cause serious complications.



For a more detailed discussion, see Kavita Natarajan and Abdullah Kutlar: Disorders of Hemoglobin Structure: Sickle Cell Anemia and Related Abnormalities, Chap. 49 in *Williams Hematology,* 9th ed.

CHAPTER 18
Methemoglobinemia and Other Dyshemoglobinemias

DEFINITION

- Increased methemoglobin from a baseline of less than 1% occurs due to oxidation of ferro to ferric iron of hemoglobin from environmental agents or due to underlying germline mutations causing diminished reduction of methemoglobin to hemoglobin. Cyanosis is almost always seen.
- Dyshemoglobinemia is a term used for modified hemoglobins (eg, methemoglobin, carboxyhemoglobin, nitrosohemoglobin, and sulfhemoglobin) that are associated with normal amino acid sequence of hemoglobin tetramers; however in M hemoglobins the globins are mutated changing amino acid in hemoglobin tetramers. They can result in varying degree of clinical manifestations.

METHEMOGLOBINEMIA

Toxic Methemoglobinemia

- Drugs or chemicals may cause methemoglobinemia either by oxidizing hemoglobin directly or by enhancing its oxidization by molecular oxygen.
- Table 18–1 lists common agents that cause methemoglobinemia.
- Infants are more susceptible to acquired toxic methemoglobinemia after prototypical ingestion of well water containing nitrites because of low levels of an enzyme that converts methemoglobin to hemoglobin (cytochrome b_5 reductase) in the newborn period. A syndrome of diarrhea, acidosis, and methemoglobinuria of yet unexplained etiology can be seen in infancy.
- Severe acute methemoglobinemia impairs oxygen delivery, and levels exceeding 30% can be fatal.
- Chronic methemoglobinemia is usually asymptomatic, but at levels greater than 20%, mild erythrocytosis is often present.
- Treatment with intravenous methylene blue (given at 1 to 2 mg/kg over 5 minutes) is rapidly effective. Excessive amounts of methylene blue, or its use in patients with glucose-6-phosphate dehydrogenase deficiency, can cause acute hemolysis.

TABLE 18–1 SOME DRUGS THAT CAUSE METHEMOGLOBINEMIA

Phenazopyridine (Pyridium)
Sulfamethoxazole
Dapsone
Aniline
Paraquat/monolinuron

Nitrate
Nitroglycerin
Amyl nitrite
Isobutyl nitrite
Sodium nitrite
Benzocaine
Prilocaine
Methylene blue
Chloramine

Source: *Williams Hematology*, 9th ed, Chap. 50, Table 50–1.

Cytochrome b_5 Reductase Deficiency

- Cytochrome b_5 reductase (also known as reduced nicotinamide [NADH] diaphorase) catalyzes the reduction of cytochrome b_5, which, in turn, reduces methemoglobin to hemoglobin.
- Heterozygosity for cytochrome b_5 reductase deficiency are usually not clinically significant but may predispose to toxic methemoglobinemia.
- Homozygosity of cytochrome b_5 reductase deficiency leads to methemoglobinemia and if restricted to erythrocytes, cyanosis is the only phenotype (type I cytochrome b_5 reductase deficiency). This is seen sporadically in all racial groups but is reported to be endemic in native Siberian ethnic groups of Yakutsk region, Navajo Indians, and Athabascan natives of Alaska.
- In some subjects, cytochrome b_5 reductase mutations are seen in all cells (not restricted to erythrocytes), and a less common hereditary syndrome of cyanosis with mental retardation and other developmental defects may occur (type II cytochrome b_5 reductase deficiency).
- Methemoglobin levels vary between 8% and 40%, and the cytochrome b_5 reductase level is less than 20% of normal.
- Treatment with ascorbic acid (200 to 600 mg/d orally, divided into four doses) lowers the methemoglobin level but is of cosmetic benefit only (reduces cyanosis).

Cytochrome b_5 Deficiency

- Rarely, cytochrome b_5 itself is deficient, causing the same clinical picture as type II cytochrome b_5 reductase deficiency.

M Hemoglobins

- Some amino acid substitutions in hemoglobin lead to enhanced formation and inability to reduce methemoglobin. These abnormal proteins are termed *M hemoglobins* (present in heterozygous state; homozygosity not described), and the resultant cyanosis from methemoglobinemia is inherited as a dominant disorder.
- Cyanosis may be evident at birth in hemoglobin M disease with the α-chain mutant; in the β-chain variant, this will evolve over 6 to 9 weeks as γ-globin chains are replaced by β-chains.
- No effective treatment for methemoglobinemia due to hemoglobin M is known.
- The characteristics of M hemoglobins are shown in **Table 18–2**.

TABLE 18–2 PROPERTIES OF M HEMOGLOBINS

Hemoglobin	Amino Acid Substitution	Oxygen Dissociation and Other Properties	Clinical Effect
Hgb M_{Boston}	α58 (E7)His → Tyr	Very low oxygen affinity, almost nonexistent heme–heme interaction, no Bohr effect	Cyanosis resulting from formation of methemoglobin
Hgb $M_{Saskatoon}$	β63 (E7)His → Tyr	Increased oxygen affinity, reduced heme–heme interaction, normal Bohr effect, slightly unstable	Cyanosis resulting from methemoglobin formation, mild hemolytic anemia exacerbated by ingestion of sulfonamides
Hgb M_{Iwate}	α87 (F8)His → Tyr	Low oxygen affinity, negligible heme–heme interaction, no Bohr effect	Cyanosis resulting from formation of methemoglobin
Hgb $M_{HydePark}$	β92 (F8)His → Tyr	Increased oxygen affinity, reduced heme interaction, normal Bohr effect, slightly unstable	Cyanosis resulting from formation of methemoglobin, mild hemolytic anemia
Hgb $M_{Milwaukee}$	β67 (E11)Val → Glu	Low oxygen affinity, reduced heme–heme interaction, normal Bohr effect, slightly unstable	Cyanosis resulting from methemoglobin formation
Hgb FM_{Osaka}	$^{G}\gamma$63His → Tyr	Low oxygen affinity, increased Bohr effect. Methemoglobinemia	Cyanosis at birth
Hgb $FM_{FortRipley}$	$^{G}\gamma$92His → Tyr	Slightly increased oxygen affinity	Cyanosis at birth

Source: *Williams Hematology,* 9th ed, Chap. 50, Table 50–2.

LOW OXYGEN AFFINITY HEMOGLOBINS

- Some hemoglobin variants have a decreased oxygen affinity, and therefore, an increased proportion of the hemoglobin is not oxygenated.
- The result may be cyanosis and mild anemia, the latter resulting from the fact that the body perceives adequate oxygen delivery and erythropoietin levels are therefore decreased.
- **Table 18–3** gives features and effects of low oxygen affinity hemoglobins.

TABLE 18–3 EXAMPLES OF LOW-AFFINITY HEMOGLOBINS

Hemoglobin	Amino Acid Substitution	Oxygen Dissociation and Other Properties	Clinical Effect
$Hgb_{Seattle}$	β70 (E14)Ala → Asp	Decreased oxygen affinity normal heme-heme interaction	Mild chronic anemia associated with reduced urinary erythropoietin; physiologic adaptation to more efficient oxygen release to tissues
Hgb_{Kansas}	β102 (G4)Asn → Thr	Very low oxygen affinity, low heme-heme interaction, dissociates into dimers in ligand form	Cyanosis resulting from deoxyhemoglobin, mild anemia

Source: *Williams Hematology,* 9th ed, Chap. 50, Table 50–3.

SULFHEMOGLOBIN

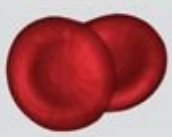
- In vitro sulfhemoglobin can be produced by addition of hydrogen sulfide to hemoglobin.
- In vivo sulfhemoglobin can be induced in some individuals by ingestion of drugs or may occur without apparent cause.
- Cyanosis is present, and occasionally mild hemolysis occurs. Sulfhemoglobinemia is usually well tolerated and does not affect overall health. Sulfhemoglobin cannot be converted back to normal hemoglobin.

CARBOXYHEMOGLOBIN

- Carbon monoxide (CO) is an odorless, colorless, and tasteless gas. It can be unknowingly inhaled to dangerous levels when present in high concentration in the atmosphere.
- Acute CO intoxication is one of the most common causes of morbidity as a result of poisoning in the United States.
- Sign and symptoms of CO poisoning are nonspecific. A high index of suspicion should attend the simultaneous presentation of multiple patients from the same housing complex. Common symptoms of mild to moderate CO poisoning are irritability, headache, nausea, lethargy, and sometimes a flu-like condition. Acute and severe poisoning can result in cerebral edema, pulmonary edema, and cardiac arrhythmias that may be deadly, and significant residual neurologic deficits may remain in survivors.
- The most important step in the treatment for CO poisoning is prompt removal of patients from the source of CO (for mild to moderate cases of CO poisoning) followed by administering 100% supplemental oxygen via a tight-fitting mask (in severe cases of CO poisoning).

NITRIC OXIDE AND NITRIC OXIDE–HEMOGLOBIN

- Nitric oxide (NO), a soluble gas, is continuously synthesized in endothelial cells by isoforms of the NO synthase enzyme. Vasodilation is caused by diffusion of NO into the smooth muscle cells.
- According to the S-nitroso hemoglobin (SNO-Hb) hypothesis, this vasodilator function is carried by a population of hemoglobin that have undergone the addition of NO to a critical cysteine (cysβ93) via S-nitrosylation, forming SNO-Hb. The allosterically controlled equilibrium of NO groups between hemes and cysteine thiols enables erythrocytes to convey a graded signal for vasodilatation, thereby enhancing perfusion.
- Another mechanism by which hemoglobin may be converted to SNO-Hb is by hemoglobin function as nitrite reductase. Deoxygenated hemoglobin reacts with nitrite to form NO and methemoglobin. Products of the nitrite-hemoglobin reaction generate NO, promote vasodilation and form SNO-Hb.



For a more detailed discussion, see Archana Agarwal and Josef T. Prchal: Methemoglobinemia and Other Dyshemoglobinemias, Chap. 50, p. 789 in *Williams Hematology*, 9th ed.

CHAPTER 19
Fragmentation Hemolytic Anemia

DEFINITION

- Acquired erythrocyte fragmentation occurs when red cells are forced at high shear stress through partial vascular occlusions or over abnormal vascular surfaces.
- In circumstances in which fragmentation of red cells occurs in the microcirculation, it is often referred to as microangiopathic hemolytic anemia.
- In three primary disorders—thrombotic thrombocytopenic purpura (TTP) (Chap. 90), hemolytic uremic syndrome (HUS) (Chap. 90), and disseminated intravascular coagulation (DIC) (Chap. 85)—microangiopathic (fragmentation) hemolytic anemia is an essential diagnostic feature. These disorders are discussed in other chapters.
- In addition to signs of hemolysis such as anemia, reticulocytosis, decreased haptoglobin, elevated indirect bilirubin, and sometimes elevated serum lactic dehydrogenase, fragmented red cells (schistocytes) are evident in the blood film (**Figure 19–1**). Their prevalence may vary.
- **Fragmentation hemolytic anemia syndromes discussed in this chapter** include (1) hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome, (2) disseminated malignancy, (3) heart valve hemolysis, (4) march hemoglobinuria, and (5) the Kasabach-Merritt phenomenon.

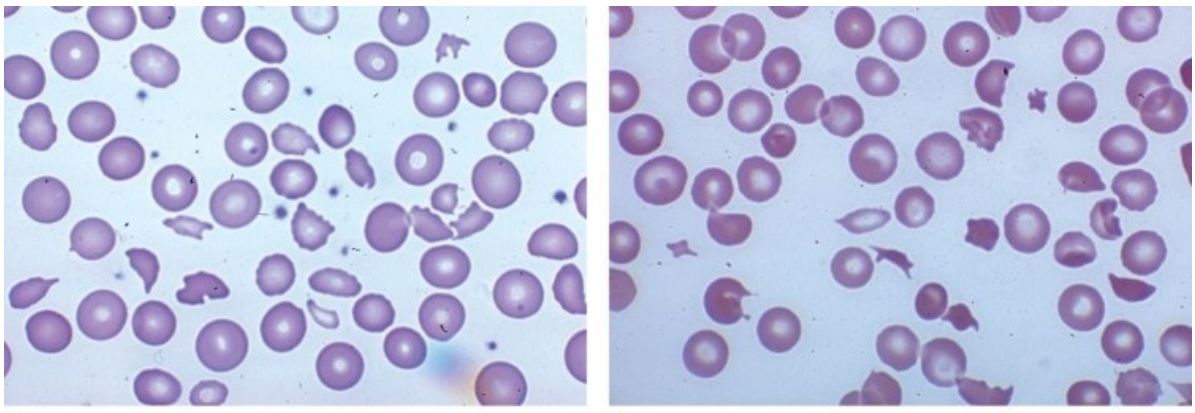


FIGURE 19–1 This image shows typical appearance of fragmented red cells. Two cases of fragmentation hemolytic anemia as a result of heart valve hemolysis. The red cell shape abnormalities are varied and characteristic of fragmentation hemolysis, although they are not specific for the cause. In the normal blood film, cells that deviate significantly in form from the normal circular shape occur only once every several thousand cells. The average oil immersion field in an area suitable for examining red cell morphology contains approximately 200 red cells. In a patient with anemia, that number may be much less. Thus, even one fragmented cell per oil immersion field is notable, although not in itself diagnostic. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

HELLP SYNDROME

- HELLP syndrome, a life-threatening condition of pregnancy, causes hemolysis, elevated liver function tests, and low platelets.

Etiology and Pathogenesis

- Abnormal development of the placental vasculature causes increased vascular tone, hypertension, proteinuria, enhanced platelet activation and aggregation, and decreased levels of the vasodilators prostaglandin I_2 and nitrous oxide.
- Concurrent activation of the coagulation cascade results in platelet-fibrin deposition in the capillaries, multiorgan microvascular injury, microangiopathic (fragmentation) hemolytic anemia, elevated liver enzymes because of hepatic necrosis, and thrombocytopenia because of peripheral consumption.
- Risk factors for HELLP syndrome include European ancestry, multiparity, advanced maternal age (> 34 years), and a personal or familial history of the disorder.
- Homozygosity for the 677C → T polymorphism of the methylenetetrahydrofolate reductase gene is a modest risk factor for the development of preeclampsia but is not associated with the development of HELLP syndrome. Whether or not the factor V Leiden or prothrombin 20210 gene mutations are risk factors for HELLP syndrome remains controversial.

Clinical Features

- Two thirds of patients are diagnosed antepartum, usually between 27 and 37 weeks. The remaining one third are diagnosed postpartum, from a few to 48 hours following delivery (occasionally as long as 6 days).
- Ninety percent of patients will present with malaise and right upper quadrant or epigastric pain.
- About 50% of patients will have nausea, vomiting, edema, or headache.
- Although hypertension is found in 85% of affected patients, 15% will not develop either hypertension or proteinuria.
- In patients with severe liver involvement, hepatic ultrasonography shows areas of increased echogenicity. As the disease progresses, large areas of necrosis can coalesce and dissect into the liver capsule. This produces a subcapsular hematoma and the risk of hepatic rupture.
- Maternal death occurs in 3% to 5% of those with HELLP syndrome and can be due to cerebral hemorrhage, cardiopulmonary arrest, DIC, adult respiratory distress syndrome, hypoxic ischemic encephalopathy, infection, placenta abruptio, postpartum hemorrhage, intra-abdominal bleeding, and subcapsular liver hematomas with resultant rupture.
- Renal complications include acute renal failure, hyponatremia, and nephrogenic diabetes insipidus.
- Pulmonary complications consist of pleural effusions, pulmonary edema, and adult respiratory distress syndrome.
- Neurologic sequelae not mentioned above are retinal detachment, postictal cortical blindness, and hypoglycemic coma.
- Fetal morbidity and mortality occurs in 9% to 24% of cases due to prematurity, placental

abruption, intrauterine asphyxia, and intraventricular hemorrhage.

Laboratory Features

- In approximately two thirds of patients, the blood film has schistocytes, helmet cells, and burr cells consistent with microangiopathic hemolytic anemia.
- Reticulocytosis can be present.
- Low haptoglobin levels are both sensitive and specific haptoglobin level is sensitive for confirming hemolysis and return to normal 24 to 30 hours postpartum.
- Lactate dehydrogenase (LDH) level is increased, due most likely to liver damage rather than hemolysis.
- Serum levels of aspartic acid transaminase (AST) and alanine transaminase (ALT) can be more than 100 times normal, whereas alkaline phosphatase values are typically only about twice normal and total bilirubin ranges between 1.2 and 5 mg/dL. Liver enzymes usually return to baseline within 3 to 5 days postpartum.
- The more severe the thrombocytopenia, the greater the risk of bleeding, perinatal and maternal morbidity and mortality, and disease recurrence with subsequent pregnancies.
- The prothrombin time (PT) and activated partial thromboplastin time (aPTT) are usually within normal limits.
- Low fibrinogen levels are found inconsistently.
- Values of von Willebrand factor (VWF) antigen increase in proportion to the severity of the disease, reflecting the extent of endothelial damage; however, no unusually large VWF multimers are present in plasma. ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin domains-13) levels are within a broad normal range (ADAMTS13 normally declines moderately during pregnancy).

Differential Diagnosis

- Other complications of pregnancy that can be confused with HELLP include TTP, HUS, sepsis, DIC, connective tissue disease, antiphospholipid antibody syndrome, and acute fatty liver of pregnancy.
- Acute fatty liver of pregnancy is seen in the last trimester or postpartum and presents with thrombocytopenia and right upper quadrant pain, but the levels of AST and ALT are usually only 1 to 5 times above normal and the PT and PTT are both prolonged.
- Because it causes right upper quadrant pain and nausea, HELLP has been provisionally misdiagnosed as viral hepatitis, biliary colic, esophageal reflux, cholecystitis, and gastric cancer.

Treatment

- Supportive care includes intravenous administration of magnesium sulfate to control hypertension and prevent eclamptic seizures, stimulation of fetal lung maturation with beclomethasone, and delivery of the fetus as soon as possible.
- Postpartum uterine curettage can lower the mean arterial pressure and increase the urine output and platelet count.
- Transfusion therapy with packed red cells, platelets, or fresh-frozen plasma is indicated in

cases complicated by severe anemia or bleeding because of coagulopathy.

- Dexamethasone is no longer used because large randomized trials found that it did not reduce the duration of hospitalization, amount of blood products transfused, maternal complications, or time to normalization of laboratory abnormalities.
- Plasma exchange cannot arrest or reverse HELLP syndrome when used antepartum, but it may minimize hemorrhage and morbidity peripartum. It can also be tried postpartum in the 5% of patients who fail to improve within 72 to 96 hours of delivery.
- Liver transplantation may be necessary in occasional patients with large hepatic hematomas or total hepatic necrosis.

HEMOLYSIS DUE TO DISSEMINATED MALIGNANCY

Etiology and Pathogenesis

- Cancer-associated microangiopathic hemolytic anemia (MAHA) has been described in a wide variety of malignancies and is more likely to occur with metastatic mucinous adenocarcinomas than with localized cancers or benign tumors.
- MAHA by either intravascular tumor emboli or DIC with intravascular occlusions of small vessels by platelet-fibrin thrombi.
- Table 19–1 lists the metastatic cancers most frequently associated with red cell fragmentation.

TABLE 19–1 CANCER ASSOCIATED WITH MICROANGIOPATHIC HEMOLYTIC ANEMIA

Gastric (55%)
Breast (13%)
Lung (10%)
Other adenocarcinomas
Unknown primary
Prostate
Colon
Gallbladder
Pancreas
Ovary
Other malignancies
Hemangiopericytoma
Hepatoma
Melanoma
Small cell cancer of the lung
Testicular cancer
Squamous cell cancer of the oropharynx
Thymoma
Erythroleukemia

Source: *Williams Hematology*, 9th ed., Chap. 51, Table 51–1.

Clinical Features

- MAHA caused by cancer is usually a preterminal event. Life expectancy following diagnosis is 2 to 150 days, with a mean of 21 days.

Laboratory Features

- Patients present with moderate to severe anemia.
- The blood film reveals schistocytes, burr cells, microspherocytes, reticulocytes, polychromasia, and nucleated red cells.
- Although the reticulocyte count can be high, it is an unreliable measure of hemolysis because extensive replacement of the marrow by metastatic tumor and cancer caused anemia of chronic disease (Chap. 5) may prevent the reticulocytosis expected.
- Absent or low levels of haptoglobin may be seen; however, it is also unreliable because haptoglobin is an acute phase reactant that can increase in malignancy.
- The mean platelet count among affected patients is approximately 50×10^9/L (range: 3–225×10^9/L). Some patients with malignant tumors may have preexisting thrombocytosis, and so superimposed MAHA may reduce the platelet count only toward "normal" values.
- Leukoerythroblastosis caused by marrow invasion, along with MAHA, is highly suggestive of metastatic malignancy.
- Laboratory evidence of DIC has been reported in approximately 50% of patients with MAHA secondary to malignancy.

Differential Diagnosis

- The most common cause of anemia in malignancy is anemia of chronic disease-inflammation (Chap. 5).
- Also consider blood loss, myelophthisis as a result of disease metastatic to the marrow (Chap. 12), and autoimmune hemolytic anemia (Chap. 22). The latter is more often found with lymphoproliferative disease but is occasionally seen with carcinoma of the stomach, colon, breast, and cervix.
- Drug treatment of cancer can also induce anemia by causing myelosuppression, oxidative hemolysis (doxorubicin, pentostatin), autoimmune hemolysis, or thrombotic microangiopathic anemia (mitomycin C, cisplatin, gemcitabine, and targeted cancer agents).

Treatment

- Heparin, glucocorticoids, dipyridamole, indomethacin, and ε-aminocaproic acid have all been tried without proven success.
- Transfusion of plasma, platelets, and cryoprecipitate may be useful during bleeding episodes associated with prolonged PT and PTT times, low fibrinogen levels, and thrombocytopenia.
- Control of the underlying malignancy can be beneficial.

HEART VALVE HEMOLYSIS

Etiology and Pathogenesis

- Anemia arising after cardiac valve replacement is caused by erythrocyte shearing and fragmentation as the red cells traversed the turbulent flow through or around the prosthetic valve.
- The incidence of significant valve-associated hemolysis is currently less than 1% with newer-generation prostheses.
- Hemolysis can be seen following mitral valve repair and in unoperated patients with native

valvular disease and hypertrophic obstructive cardiomyopathy.

- Risk factors for valvular hemolysis include central or paravalvular regurgitation, placement of small valve prostheses with resultant high transvalvular pressure gradients, regurgitation because of bioprosthetic valve failure, ball-and-cage valves, bileaflet valves versus tilting disk valves, mechanical valve prostheses versus xenograft tissue prostheses, double-valve versus single-valve replacement, and aortic versus mitral valve prostheses.

Clinical Features

- Patients with valve-induced hemolysis can present with symptoms caused by anemia or congestive heart failure, pallor, icterus, and dark urine (described variously as red, brown, or black).
- Urine excreted during periods of physical activity may be darker than that excreted at rest.
- Hemolysis can be exacerbated by supraventricular tachycardia or other tachyarrhythmias and regress once normal sinus rhythm is restored.

Laboratory Features

- The red cells on the blood film will reveal moderate poikilocytosis, schistocytosis (fragmentation), and polychromasia.
- The red cells are usually normochromic but can occasionally be hypochromic and microcytic as a result of long-standing urinary iron loss.
- **Table 19–2** lists the principal findings by severity of hemolysis in patients with prosthetic heart valves. The reticulocyte count, urine hemosiderin, plasma hemoglobin, and serum levels of total and indirect bilirubin, and LDH may be elevated, whereas the serum haptoglobin is decreased.
- Both the number of schistocytes in the blood and the elevation of LDH correlate with the severity of hemolysis.
- Hemoglobinuria is usually only seen in those with particularly severe hemolysis and high LDH levels.

TABLE 19–2 SEVERITY OF PROSTHETIC VALVE HEMOLYSIS

	Mild	Moderate	Severe
Hemosiderinuria	Present	Present	Marked
Hemoglobinuria	Absent	Absent	Absent
Schistocytosis	< 1%	> 1%	>> 1%
Reticulocytosis	< 5%	> 5%	>> 5%
Haptoglobin	Decreased	Absent	Absent
LDH	< 500 U/L	> 500 U/L	>> 500 U/L

LDH, lactate dehydrogenase.
Data from E Eyster, J Rothchild, O Mychajliw.
Source: *Williams Hematology*, 9th ed., Chap. 51, Table 51–2.

Differential Diagnosis

- Factors that can promote valve-associated hemolysis or worsen the resultant anemia include iron deficiency, because (1) anemia increases cardiac output and shear stress and (2) hypochromic red cells are more fragile than normal.
- Folate deficiency can result from the increased erythropoiesis associated with hemolysis limiting the erythropoietic compensatory response (see Chap. 8).
- Infectious endocarditis causes anemia of chronic disease.
- Anticoagulation to minimize thrombosis around mechanical heart valves can cause gastrointestinal blood loss and subsequent iron-deficiency anemia.

Treatment

- Iron and folate replacement, if deficient, may be effective.
- Surgical repair or replacement of the malfunctioning prosthesis should take place, if possible. Poor surgical candidates with perivalvular leaks may benefit from percutaneous closure with an Amplatzer occluder device.
- Adjunctive measures to be tried include β-adrenergic blockade to slow the velocity of the circulation, erythropoietin therapy to stimulate erythropoiesis, and pentoxifylline therapy (400 mg orally 3 times daily) to increase the deformability of red cells.
- Ursodeoxycholic acid, 600 mg orally once daily beginning 1 week before valve replacement surgery, significantly decreases the incidence of pigmented gallstone formation.

MARCH HEMOGLOBINURIA

- The presenting complaint is passage of dark urine immediately following physical exertion in the upright position, occasionally accompanied by nausea, abdominal cramps, aching in the back or legs, or a burning feeling in the soles of the feet.
- Hepatosplenomegaly and transient jaundice have been rarely reported.
- This condition is caused by red cell trauma within the vessels of the soles of the feet, the severity is influenced by the hardness of the running surface, the distance run, the heaviness of the athlete's stride, and the quality of one's footwear.
- Anemia is uncommon and if present is usually mild, but repeated episodes can cause iron deficiency and resultant anemia.
- Morphologic evidence of red cell damage on the blood film is usually not seen.
- Renal damage is also not commonly seen, but cases of acute tubular necrosis and resultant acute renal insufficiency have been described.

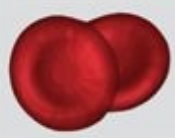
KASABACH-MERRITT PHENOMENON

- This condition usually develops in early childhood and is characterized by thrombocytopenia, microangiopathic hemolytic anemia, consumptive coagulopathy, and hypofibrinogenemia caused by an enlarging kaposiform hemangioendothelioma or tufted angiomas.
- Kaposiform hemangioendotheliomas are highly aggressive, vascular tumors that occur equally in males and females and show little tendency to resolve spontaneously. However, they have never been reported to metastasize.

- It is postulated that endothelial cell abnormalities and vascular stasis lead to activation of platelets and the coagulation cascade within the tumor's vessels, with subsequent depletion of both platelets and clotting factors.
- Microangiopathic hemolytic anemia results from mechanical trauma sustained by the erythrocytes traversing the tumor's abnormal, partially thrombosed vascular channels.
- The mortality can be as high as 30%.
- Surgical resection is always followed by normalization of hematologic parameters, but many lesions are too large to be resected without severe disfigurement.
- Other treatments include glucocorticoids, interferon-α, antifibrinolytic agents, antiplatelet agents, low-molecular-weight heparin, embolization, radiation, laser therapy, and various forms of chemotherapy.

MISCELLANEOUS DISEASES ASSOCIATED WITH FRAGMENTATION HEMOLYTIC ANEMIA

These include malignant systemic hypertension; pulmonary hypertension; giant cavernous hemangiomas of the liver; and vasculitides, including Wegener granulomatosis and giant cell arteritis.



For a more detailed discussion, see Kelty R. Baker and Joel Moake: Fragmentation Hemolytic Anemia, Chap. 51 in *Williams Hematology*, 9th ed.

CHAPTER 20

Hemolytic Anemia Resulting from a Chemical or Physical Agent

- Hemolysis can be mainly intravascular (ie, hypotonic lysis or heat damage) or predominantly extravascular (ie, arsine gas and oxygen).
- Certain drugs can induce hemolysis in individuals with abnormalities of erythrocytic enzymes, such as glucose-6-phosphate dehydrogenase, or with an unstable hemoglobin (see Chaps. 14 and 17). Such drugs can also cause hemolysis in normal individuals if given in sufficiently large doses.
- Other drugs induce hemolytic anemia through an immunologic mechanism (see Chap. 24).
- The drugs and chemicals discussed here cause hemolysis by other mechanisms.

ARSENIC HYDRIDE (ARSINE, AsH_3)

- Arsine gas (arsenic hydride is formed in many industrial processes) may lead to hemolysis.
- In some areas, the water supply can be tainted with arsenic.
- Inhalation of arsine gas can lead to severe anemia, hemoglobinuria, and jaundice as a result of the oxidation of sulfhydryl groups in the red cell membrane.
- The red cells may become spherocytic and stomatocytic and severely hypochromic from hemoglobin loss (red cell ghosts) (**Figure 20–1A**).

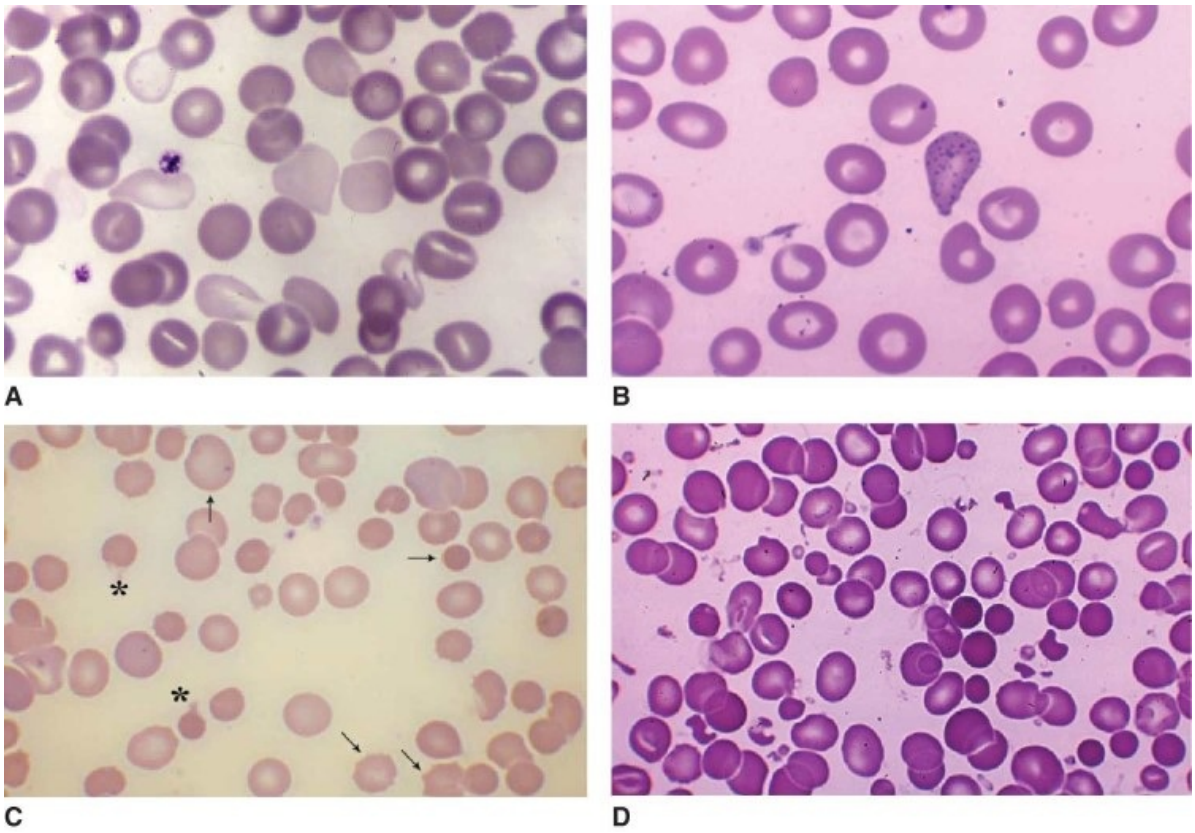


FIGURE 20–1 **A.** Blood film prepared from a patient exposed to arsenic hydride (AsH). Note the very pale red cells resulting from partial hemoglobin loss secondary to membrane damage. An extreme example, represented by the virtual ghost thinly rimmed with scant residual hemoglobin, can be found in the upper left-hand corner. Other cells are spherocytic or stomatocytic. **B.** Blood film from person with lead poisoning. Note the tear-drop–shaped red cell with basophilic stippling. The stippling may be fine or course but may be minimized or absent in blood anticoagulated with Na_2EDTA. **C.** Wilson disease. In this image from a patient with Wilson disease, there are numerous visible sequelae of oxidative damage caused by excess copper. The striking dense microspherocytes indicates damage to the membrane. Damage to hemoglobin is demonstrated by the Heinz bodies projecting from red cells (*asterisks* show two examples). The *horizontal arrow* points to one of several microspherocytes. The *vertical arrow* points to a macrocyte (reticulocyte). An occasional cell shows damage to both membrane and hemoglobin. The presence of echinocytes (*oblique arrows* show two examples) suggests that the liver is also affected. **D.** Blood film prepared at admission from a patient who had suffered severe burn injury involving a large percentage of the body surface. Note the presence of normal erythrocytes (apparently from vessels not exposed to heat damage) along with populations of normocytic and microcytic spherocytes. In addition, there are numerous red cell fragments, some smaller than platelets, from heat-related red cell fragmentation. (Images A, B, and D: Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com; Image C: Used with permission from Barbara J. Bain, Imperial College, London, UK.)

LEAD

- Lead poisoning in children usually is a result of ingestion of lead paint flakes or chewing lead-paint coated objects. In adults, it usually is the result of industrial exposure.
- Lead poisoning may lead to abdominal pain, confusion, headache, and in severe cases seizures, coma, and death.
- Lead intoxication leads to anemia largely caused by inhibition of heme synthesis and the rate of red cell production. There is also a modest decrease in red cell life span (mild hemolysis).
- Lead also inhibits pyrimidine 5′-nucleotidase (see Chap. 14), which may be responsible for the basophilic stippling of red cells found in lead poisoning. Basophilic stippling may be fine or coarse and is often found in polychromatophilic cells (see **Figure 20–1B**).
- The anemia is usually mild in adults but may be severe in children. Red cells are normocytic

and slightly hypochromic.
- Ringed sideroblasts are frequently found in the marrow (see Chap. 11).

COPPER

- Hemolytic anemia may be induced by high levels of copper in patient's hemodialyzed with fluid contaminated by copper tubing, or it may occur in patients with Wilson disease.
- Wilson disease may present or be called to medical attention by a hemolytic anemia, often having spherocytes and Heinz bodies as a result of copper injury to red cells (see **Figure 20–1C**). The presence of liver disease with a hemolytic anemia should raise the question of Wilson disease. (See **Table 20–1** for laboratory findings in Wilson disease.)
- The hemolysis is probably caused by copper-induced inhibition of several erythrocyte enzymes and membrane injury.

TABLE 20–1 LABORATORY FINDINGS IN WILSON DISEASE

Variable	Normal Value	Wilson Disease
Serum ceruloplasmin (mg/L)	200–400	< 200
Serum copper (µM)	11–24	< 11
Urinary copper (µg/24 h)	≤ 40	> 100
Liver copper (µg/g dry weight)	20–50	> 200

CHLORATES

- Ingestion of sodium or potassium chlorate, or contamination of dialysis fluid with chloramines, can cause oxidative damage with formation of Heinz bodies and methemoglobin and with development of hemolytic anemia.

MISCELLANEOUS DRUGS AND CHEMICALS

- Other drugs and chemicals that can cause hemolytic anemia are listed in **Table 20–2**.

TABLE 20–2 DRUGS AND CHEMICALS THAT HAVE BEEN REPORTED TO CAUSE HEMOLYTIC ANEMIA

Chemicals	Drugs
Aniline	Amyl nitrite
Apiol	Mephenesin
Dichlorprop (herbicide)	Methylene blue
Formaldehyde	Omeprazole
Hydroxylamines	Pentachlorophenol
Lysol	Phenazopyridine (Pyridium)
Mineral spirits	Salicylazosulfapyridine (Azulfidine)

Nitrobenzene	Tacrolimus
Resorcin	

Source: *Williams Hematology*, 9th ed, Chap. 52, Table 52–1.

WATER

- Water administered intravenously, inhaled in near-drowning, or gaining access to the circulation during irrigation procedures can cause hemolysis.

OXYGEN

- Hemolytic anemia has developed in patients receiving hyperbaric oxygenation and in astronauts exposed to 100% oxygen.

INSECT AND ARACHNID VENOMS

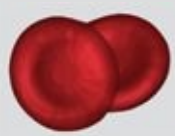
- Severe hemolysis may occur in some patients following bites by bees, wasps, spiders, or scorpions.
- Snake bites are only rarely a cause of hemolysis.

HEAT

- Patients with extensive burns may develop severe hemolytic anemia, apparently as a result of direct damage to the red cells passing through the skin and subcutaneous tissues by extreme heat.
- Blood films of many burned patients show spherocytes and fragmentation as a result of severe membrane injury. (See **Figure 20–1D**.)

NEOCYTOLYSIS

- Neocytolysis, the selective destruction of young red cells, is a phenomenon unique to microgravity and is associated with a rapid decrease in erythropoietin levels.
- It is experienced by astronauts after space flight, even in the presence of normal ambient oxygen concentration, or in people rapidly descending from high altitude to sea level.
- Radiolabeling studies of erythrocytes indicated that the anemia was caused by selective hemolysis of young erythrocytes less than 12 days old.



For a more detailed discussion, see Paul C. Herrmann: Hemolytic Anemia Resulting from Chemical and Physical Agents, Chap. 52 in *Williams Hematology*, 9th ed.

CHAPTER 21
Hemolytic Anemia Resulting from Infectious Agents

- Hemolysis represents a prominent part of the overall clinical picture in many infections. **Table 21–1** lists the microorganisms associated with the induction of hemolytic anemia.

TABLE 21–1	ORGANISMS THAT CAUSE HEMOLYTIC ANEMIA

Aspergillus
Babesia microti and *Babesia divergens*
Bartonella bacilliformis
Campylobacter jejuni
Clostridium welchii
Coxsackie virus
Cytomegalovirus
Diplococcus pneumoniae
Epstein-Barr virus
Escherichia coli
Haemophilus influenzae
Hepatitis A
Hepatitis B
Herpes simplex virus
Human immunodeficiency virus
Influenza A virus
Leishmania donovani
Leptospira ballum and/or *butembo*
Mumps virus
Mycobacterium tuberculosis
Mycoplasma pneumoniae
Neisseria intracellularis (meningococci)
Parvovirus B19
Plasmodium falciparum
Plasmodium malariae
Plasmodium vivax
Rubella virus
Rubeola virus
Salmonella
Shigella
Streptococcus
Toxoplasma
Trypanosoma brucei
Varicella virus

Vibrio cholera
Yersinia enterocolitica

Source: *Williams Hematology*, 9th ed, Chap. 53, Table 53–1.

MECHANISMS

- Hemolysis may be caused by:
  — Direct invasion of red cell by infecting organisms (*Plasmodium* sp)
  — Elaboration of hemolytic toxins (*Clostridium perfringens*)
  — Development of autoantibodies against red blood cell antigens (*Mycoplasma pneumoniae*)

MALARIA

Plasmodium Species and Severity of Anemia

- Five species of the genus *Plasmodium* cause human malaria: *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale,* and *Plasmodium knowlesi*.
- *P falciparum* and *P vivax* cause the most cases worldwide and are principally associated with hemolytic anemia.
- *P vivax* invades only young red cells.
- *P falciparum* attacks both young and old cells, and anemia tends to be more severe with this form of malaria and is the most deadly type.

Etiology and Pathogenesis

- Malaria is the world's most common cause of hemolytic anemia.
- The disease is transmitted by the bite of an infected female *Anopheles* mosquito.
- Parasites grow intracellularly, and parasitized red cells are destroyed in the spleen.
- Uninvaded red cells are also destroyed.
- Erythropoietin is low for degree of anemia secondary to release of inhibitory cytokines, especially in *P falciparum* infection.
- Prevalence of certain heterozygous mutations that interfere with invasion of red blood cells by parasites has evolved in endemic areas (eg, glucose-6-phosphate dehydrogenase [G6PD] deficiency, thalassemia, sickle cell trait, other hemoglobinopathies, and hereditary elliptocytosis).

Hemolytic Mechanisms

- Destruction of parasitized red cells occurs largely in the spleen.
- Splenomegaly typically is present in chronic malarial infection.
- "Pitting" of parasites from infected erythrocytes may occur in the spleen.
- Degree of red cell parasitemia, in part, determines the destruction of infected erythrocytes.
- Low rates of parasitemia may not contribute to the development of anemia; high rates (eg, 10%) may have very significant effects.
- The degree to which anemia develops is disproportionate to the number of cells infected with

the parasite. For each infected red cell, ten times the number of uninfected red cells are removed, magnifying the hemolytic rate.

- Hemin accumulation facilitates hemolytic cell loss via a process of programmed cell death, referred to as eryptosis.
- Activation of hepatosplenic macrophages enhances red cell clearance as a result of red cell surface changes in both parasitized and nonparasitized cells that foster recognition and erythrophagocytosis by macrophages.
- Both marked loss of red cell deformability and deposition of immunoglobulin (IgG) and complement (C3d), sometimes resulting in a positive direct antiglobulin reaction, may enhance red cell removal by macrophages.
- Splenomegaly further enhances red cell removal from the circulation.
- *P falciparum* decreases the erythropoietin response.
- Lower reticulocyte count for degree of anemia.
- Coincidental dyserythropoiesis occurs with stippling, cytoplasmic vacuolization, nuclear fragmentation, and multinuclearity.
- Inhibition of the erythroid response (anemia of chronic disease) is secondary to release of interferon-γ and tumor necrosis factor-α.

Clinical Features

- Febrile paroxysms are characteristically cyclic: *P falciparum* every 24 hours and *P vivax* every 48 hours.
- Rigors, headache, abdominal pain, nausea and vomiting, and extreme fatigue accompany the fever.
- Splenomegaly typically is present in chronic infection.
- Falciparum malaria is associated rarely with very severe hemolysis and dark, almost black urine (blackwater fever).
- Cerebral malaria may result in delirium and other neurologic manifestations.
- Organ dysfunction (respiratory insufficiency and renal failure) may be present.

Laboratory Features

- Signs of hemolytic anemia are apparent.
- Thrombocytopenia is nearly always present.
- Diagnosis of malaria depends on **one** of the following:
  — Demonstration of the parasites on the blood film **(Figure 21–1)**
  — Presence of antigenic parasite proteins using rapid detection test (RDT)
  — Polymerase chain reaction (PCR) to demonstrate the appropriate DNA sequences in the blood
  — Use of automated hematology analyzers to identify parasites as part of a standard complete blood count
- Morphologic differentiation of *P falciparum* from other forms of malaria, principally *P vivax*, is important because *P falciparum* infection may constitute a clinical emergency.
  — If more than 5% of the red cells contain parasites, the infection is almost certainly with *P falciparum*.

— *P falciparum* infection ring forms are virtually the only form of parasite evident on the blood film.
— The finding of two or more rings within the same red cells is regarded as indicative of *P falciparum* infection.
— In nonimmune patients, examination of the blood film for malarial parasites should be made for at least 3 days after onset of symptoms; parasitemia may not reach detectable levels for several days.

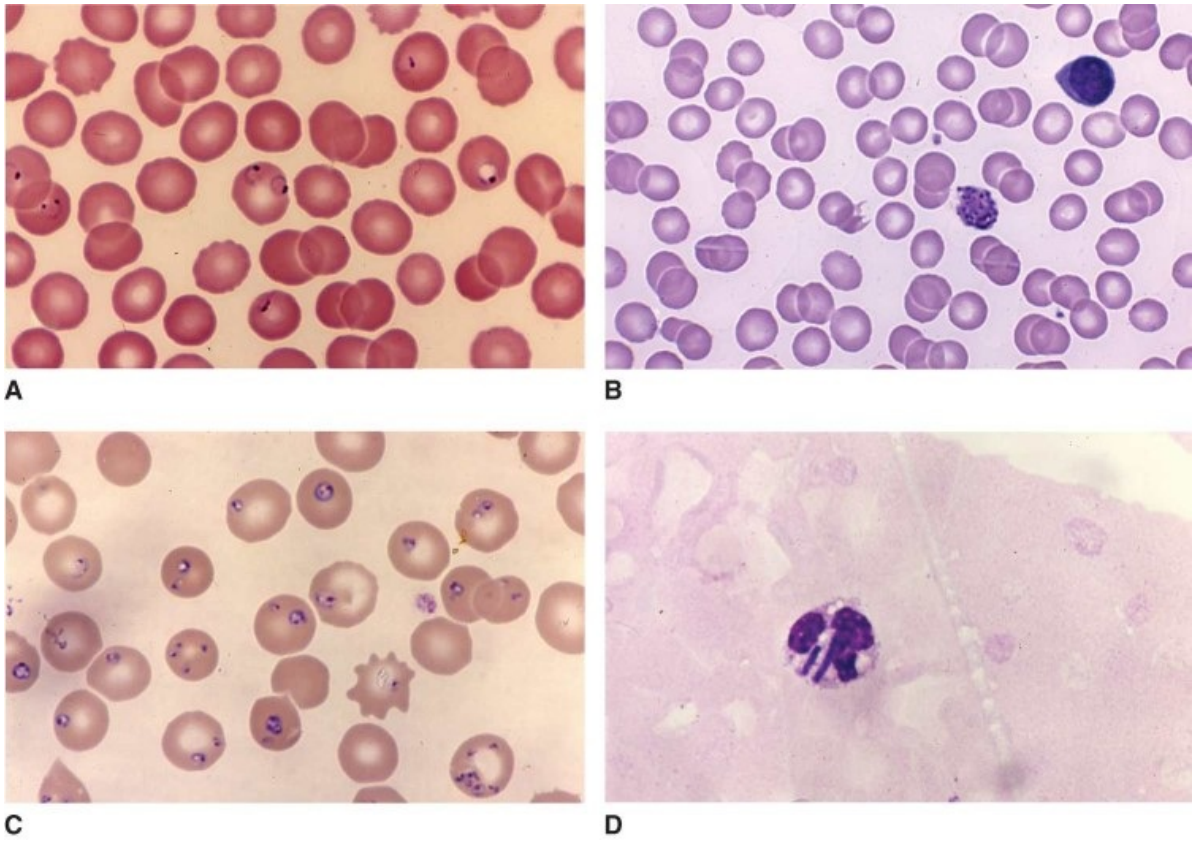


FIGURE 21–1 **A.** Blood film from a patient with malaria caused by *Plasmodium falciparum*. Several red cells contain ring forms. Note red cell with double ring form in center of the field, characteristic of *P falciparum* infection. Note the ring form with double dots at the left edge of figure, suggestive of *P falciparum* infection. Note also high rate of parasitemia (~10% of red cells in this field) characteristic of *P falciparum* infection. **B.** Blood film from a patient with malaria caused by *Plasmodium vivax*. Note mature schizont. **C.** Blood film from a patient with *Babesia microti* infection. The heavy parasitemia is characteristic of babesiosis (about two-thirds of red cells infected). **D.** Blood film from a patient with *Clostridium perfringens* septicemia. Few red cells evident as a result of intense erythrolysis by clostridial exotoxin. Neutrophil with two bacilli (*C perfringens*). (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

Treatment and Prognosis

- The blood form of malaria should be treated as soon as possible.
- Artemisinins are effective against *P falciparum*, but numerous studies are in progress to determine efficacy of individual drugs and drug combinations.
- Severity of disease and prognosis is independent of magnitude of parasitemia.
- Tissue stages of vivax malaria have been treated with primaquine. Primaquine, as well as certain sulfones, may produce severe hemolysis in patients with G6PD deficiency.
- Transfusions may be necessary in treatment of severe blackwater fever, and if renal failure occurs, dialysis may be required.
- In patients with severe malaria, cerebral malaria, or high levels of parasitemia,

erythrocytapheresis or erythrocyte exchange may be beneficial.
- With early treatment, prognosis is excellent. When therapy is delayed or the strain is resistant, malaria (particularly falciparum) may be fatal.
- Development of vaccines to prevent malaria are under intense study.

BARTONELLOSIS (OROYA FEVER)

- *Bartonella bacilliformis* is transmitted by the sand fly.
- The organism adheres to the exterior surface of red blood cells, which are rapidly removed from the circulation by the spleen and liver.

Clinical Features

- Disease develops in two stages:
  — Acute hemolytic anemia (Oroya fever)
  — Chronic granulomatous disorder (verruca peruviana)
- Most patients manifest no other clinical symptoms during the Oroya fever phase, but some may develop severe hemolytic anemia accompanied by anorexia, thirst, sweating, and generalized lymphadenopathy. Severe thrombocytopenia may occur.
- Verruca peruviana is a nonhematologic disorder characterized by bleeding, warty reddish-purple nodules over the face and extremities.

Laboratory Features

- Severe anemia develops rapidly.
- Large numbers of nucleated red cells appear in the blood and the reticulocyte count is elevated.
- Diagnosis is established by demonstrating the organisms on the surface of red cells on a Giemsa-stained smear (red-violet rods 1–3 μm in length).

Treatment and Prognosis

- Mortality in untreated patients is very high. Those who survive experience sudden clinical improvement with increase in red cell count and change of the organisms from an elongated to a coccoid form.
- The acute phase usually responds to treatment with ciprofloxacin, chloramphenicol, and β-lactam antibiotics or combinations of the aforementioned, especially in children.

BABESIOSIS

Epidemiology

- Babesiosis is most common in the northeastern coastal and Great Lakes regions where it became known as “Nantucket fever.” It also occurs in midwestern regions.
- The disease is being recognized with increasing frequency.
- *Babesia* species are intraerythrocytic protozoans known as piroplasms.

- The parasites are transmitted by ticks that may infect many species of wild and domestic animals.
- Humans occasionally become infected with *Babesia microti* (North America) or *Babesia divergens* (Europe), species that normally parasitize rodents, and, deer, elk, and cattle, respectively.
- *B divergens* usually occur in splenectomized patients. This is not the case with *B microti*.
- Other *Babesia*-like piroplasms, such as *Babesia* WA1 and *Babesia* MO1, may also produce human disease.
- The parasite is usually tick-borne in humans but has also been transmitted by transfusion.
- The risk of transfusion-transmitted babesiosis is higher than generally appreciated and in endemic areas represents a threat to the blood supply.

Clinical Features

- Symptoms are caused by reproduction of the organisms in the red cell and subsequent cell lysis.
- Clinical expression is broad, reflecting the degree of parasitemia.
- Incubation period ranges from 1 week to 3 months but usually is about 3 weeks.
- Onset is gradual, with malaise, anorexia, and fatigue, followed by fever (sometimes as high as 40°C [104°F]), chills, sweats, and muscle and joint pains.
- Onset may be fulminant. Hepatic and splenic enlargement may be evident.
- Moderate degree of hemolytic anemia is usually present; this has been sufficiently severe to cause hypotension. Transfusion is occasionally required.
- Hemolysis may last a few days, but in asplenic, elderly, or otherwise immunocompromised patients, can last for months.
- Elevation in serum transaminases, lactic dehydrogenase, unconjugated bilirubin, and alkaline phosphatase correlates with the severity of the parasitemia.
- Thrombocytopenia and leukopenia may occur.

Diagnosis

- History of exposure to a tick-infested area, recent blood transfusion, or asplenia may be significant.
- Darkly stained ring forms (*Babesia*) with light blue cytoplasm in the red cells in Giemsa-stained thin blood films are present (Figure 21–1).
- Merozoites may also be visible.
- Infrequently, an intraerythrocytic structure consisting of four daughter cells of *Babesia* connected by cytoplasmic bridges, resembling a Maltese cross, may be observed.
- The parasitemia can be high, affecting more than 75% of red cells.
- Immunofluorescent tests for antibodies to *Babesia* are available.
- PCR-based diagnostic tests are the test of choice for confirmation of an active infection in an individual bearing antibodies and for following the response to therapy.
- The onset of fever and hemolytic anemia after transfusion should lead to the consideration of babesiosis.

Treatment and Course

- Most mild *B microti* infections respond without treatment.
- Infection has responded to drug therapy with clindamycin and quinine.
- Combination of atovaquone and azithromycin has also been proposed as treatment.
- Whole blood or red cell exchange can result in marked improvement in recalcitrant cases.

Coinfection

- Two or more parasites may coinfect an individual by a tick bite (eg, *B microti and Borrelia burgdorferi* [Lyme disease] in endemic areas).
- These enter the circulation as a result of the *Ixodes* tick bite (as can other blood cell parasites [eg, human granulocytic anaplasmosis, formerly called human granulocytic ehrlichiosis, caused by *Anaplasma phagocytophilum*, which invade only neutrophils]).
- Signs and symptoms may be similar to solitary *Babesia* infection.
- Successful early treatment for Lyme disease may result in a residual *B microti* infection.
- Antibiotic therapy for Lyme disease will not eradicate *B microti*.

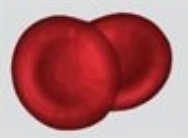
CLOSTRIDIUM PERFRINGENS (welchii)

- Infection is most common in patients with septic abortion and occasionally seen following acute cholecystitis.
- In *C perfringens* septicemia, the toxin (a lecithinase) reacts with red blood cell lipids, leading to severe, often fatal hemolysis with striking hemoglobinemia and hemoglobinuria; serum may be a brilliant red and the urine a dark-brown mahogany color.
- Acute renal and hepatic failure usually develops.
- The blood film shows microspherocytosis, leukocytosis with a left shift, and thrombocytopenia and occasionally intracellular gram-positive rods (Figure 21–1).
- The hematocrit may approach zero, but the blood (plasma) hemoglobin may be about 60 to 100 g/L at the time of acute massive intravascular hemolysis.
- Treatment is with intravenous fluid support, high-dose penicillin or a similar antibiotic (eg, ampicillin), and surgical debridement.
- Mortality is greater than 50%, even with appropriate therapy.

OTHER INFECTIONS

- Viral agents may be associated with autoimmune hemolysis (see Chap. 22). The mechanisms include absorption of immune complexes, cross-reacting antibodies, and loss of tolerance.
- Evidence for cytomegalovirus infection is found in a high percentage of children with lymphadenopathy and hemolytic anemia.
- High cold agglutinin titer may develop with *Mycoplasma pneumoniae* infection and occasionally results in hemolytic anemia or compensated hemolysis (see Chap. 23).
- *Microangiopathic hemolytic anemia* (see Chap. 19) may be triggered by a variety of infections, including *Shigella*, *Campylobacter*, and *Aspergillus*.
- Thrombotic microangiopathy with fragmentation hemolytic anemia (hemolytic uremic

syndrome), especially in children, can be caused by enterotoxigenic gram-negative microorganisms, notably *Escherichia coli* serotype O157:H7 (see Chap. 19).



For a more detailed discussion, see Marshall A. Lichtman: Hemolytic Anemia Resulting from Infections with Microorganisms, Chap. 53 in *Williams Hematology*, 9th ed.

CHAPTER 22
Hemolytic Anemia Resulting from Warm-Reacting Antibodies

- In autoimmune hemolytic anemia (AHA), shortened red blood cell (RBC) survival is the result of host antibodies that react with autologous RBC.
- AHA may be classified by whether an underlying disease is present (secondary) or not (primary or idiopathic) (**Table 22–1**).
- AHA may also be classified by the nature of the antibody (**Table 22–2**).
- "Warm-reacting" antibodies are usually of the immunoglobulin G (IgG) type, have optimal activity at 37°C, and bind complement.
- "Cold-reacting" antibodies show affinity at lower temperatures (see Chap. 23).
- Occasionally, mixed disorders occur with both warm and cold antibodies.
- Warm antibody AHA is the most common type.

TABLE 22–1 CLASSIFICATION OF WARM-ANTIBODY–MEDIATED AUTOIMMUNE HEMOLYTIC ANEMIA (AHA)

I. On basis of presence or absence of underlying or significantly associated disorder
  A. Primary or idiopathic AHA (no apparent underlying disease)
  B. Secondary AHA
    1. Associated with lymphoproliferative disorders (eg, Hodgkin or non-Hodgkin lymphoma)
    2. Associated with the rheumatic disorders, particularly systemic lupus erythematosus
    3. Associated with certain infections (eg, *Mycoplasma pneumoniae*)
    4. Associated with certain nonlymphoid neoplasms (eg, ovarian tumors)
    5. Associated with certain chronic inflammatory diseases (eg, ulcerative colitis)
    6. Associated with ingestion of certain drugs (eg, α-methyldopa)

TABLE 22–2 MAJOR REACTION PATTERNS OF THE DIRECT ANTIGLOBULIN TEST AND ASSOCIATED TYPES OF IMMUNE INJURY

Reaction Pattern	Type of Immune Injury
IgG alone	Warm-antibody autoimmune hemolytic anemia Drug-immune hemolytic anemia: hapten drug adsorption type or autoantibody type
Complement alone	Warm-antibody autoimmune hemolytic anemia with subthreshold IgG deposition Cold-agglutinin disease Paroxysmal cold hemoglobinuria Drug-immune hemolytic anemia: ternary complex type
IgG plus complement	Warm-antibody autoimmune hemolytic anemia Drug-immune hemolytic anemia: autoantibody type (rare)

Source: *Williams Hematology*, 9th ed, Chap. 54, Table 54–4.

ETIOLOGY AND PATHOGENESIS

- AHA occurs in all age groups, but the incidence rises with age, in part because the frequency

of lymphoproliferative malignancies increases with age.

- In primary AHA, the autoantibody often is specific for a single RBC membrane protein, suggesting that an aberrant immune response has occurred to an autoantigen or a similar immunogen; a generalized defect in immune regulation is not seen.
- In secondary AHA, the autoantibody most likely develops from an immunoregulatory defect.
- Certain drugs (eg, α-methyldopa) can induce specific antibodies in otherwise normal individuals by some unknown mechanism. These subside spontaneously when the drug is stopped.
- The red cells of some apparently normal individuals may be found coated with warm-reacting autoantibodies similar to those of patients with AHA. Such antibodies are noted in otherwise normal blood donors at a frequency of 1 in 10,000. A few develop AHA.
- RBC autoantibodies in AHA are pathogenic.
- RBCs that lack the targeted antigen have a normal survival in the presence of the antibody.
- Transplacental passage of autoantibodies to a fetus can cause hemolytic anemia.
- Antibody-coated RBCs are trapped by macrophages primarily in the spleen, where they are ingested and destroyed or partially phagocytosed and a spherocyte with a lower surface area-to-volume ratio is released.
- Macrophages have cell surface receptors for the Fc portion of IgG and for fragments of C3 and C4b. These immunoglobulin and complement proteins on the RBC surface can act cooperatively as opsonins and enhance trapping of RBCs.
- Large quantities of IgG, or the addition of C3b, will increase trapping of RBCs by macrophages in the liver and spleen.
- Direct RBC lysis by complement is unusual in warm antibody AHA, probably as a result of interference with complement activity by several mechanisms. Lysis by complement is seen in cold antibody type AHA and paroxysmal cold hemoglobinuria (see Chap. 23).
- RBC may be destroyed by monocytes or lymphocytes by direct cytotoxic activity, without phagocytosis. The proportion of hemolysis caused by this mechanism is unknown. Antibodies may also attach to late erythroid precursors and suppress erythropoiesis.

CLINICAL FEATURES

- Generally, symptoms of anemia draw attention to the disease, although jaundice may also be a presenting complaint.
- Symptoms are usually slow in onset, but rapidly developing anemia can occur.
- Uncommonly severe anemia may require urgent care. The patient may display air-hunger, profound pallor, and weakness. This syndrome can be seen in patients with AHA in the setting of chronic lymphocytic leukemia.
- Physical examination may be normal if the anemia is mild. Splenomegaly is common but not always observed. Jaundice and physical findings related to more pronounced anemia may be noticed.
- AHA may be aggravated or first noticed during pregnancy. Both mother and fetus generally fare well if the condition is treated early.

LABORATORY FEATURES

General

- Anemia can range from mild to life-threatening.
- Blood film reveals polychromasia (indicating reticulocytosis) and spherocytes (see **Figure 22–1**).
- With severe cases, nucleated RBC, RBC fragments, and, occasionally, erythrophagocytosis by monocytes may be seen (see **Figure 22–1C**).
- Reticulocytosis is usually present if the marrow has not been injured by some other condition, initially or if the antibody does not attach to orthochromatic erythroblasts and reticulocytes. A short period of relative reticulocytopenia occurs in one third of the cases. Glucocorticoids may permit the emergence of a reticulocytosis.
- Most patients have mild neutrophilia and normal platelet count, but occasionally immune neutropenia and thrombocytopenia can occur concomitantly.
- Evans syndrome is a condition in which both autoimmune-mediated RBC and platelet destruction occur. A low neutrophil count, as a result of immune neutropenia, may also be present.
- Marrow examination usually reveals erythroid hyperplasia; occasionally an underlying lymphoproliferative disease may be uncovered.
- Unconjugated hyperbilirubinemia is often present but may be only slightly elevated; the total bilirubin level usually does not exceed 5 mg/dL, with less than 15% conjugated.
- Haptoglobin levels are usually very low, and serum lactic acid dehydrogenase (LDH) activity often is elevated.
- Urinary urobilinogen is often increased, but hemoglobinuria is uncommon.
- The alterations in bilirubin, urobilinogen, LDH, and haptoglobin levels are related to the severity of the hemolysis.

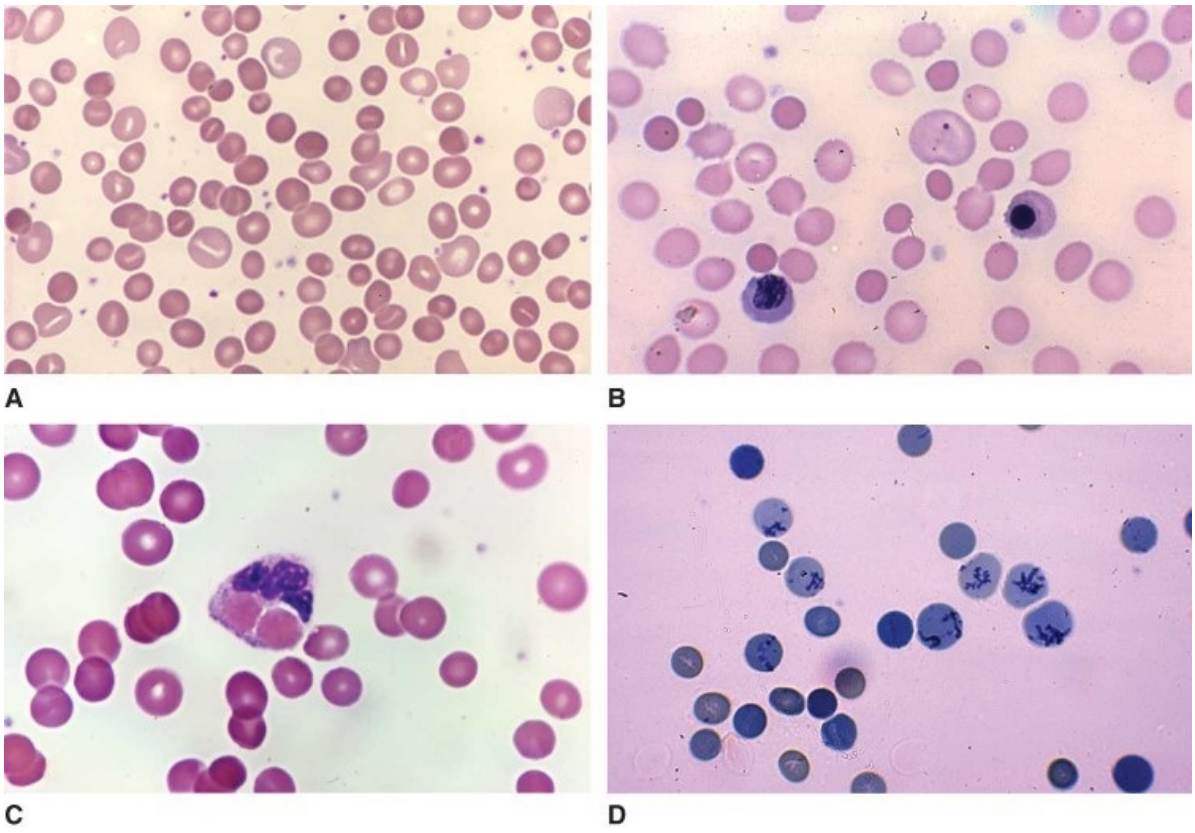


FIGURE 22–1 **A.** Blood film. Autoimmune hemolytic anemia. Moderately severe. Note high frequency of microspherocytes (small hyperchromatic red blood cells [RBCs]) and the high frequency of macrocytes (putative reticulocytes). **B.** Blood film. Autoimmune hemolytic anemia. Severe. Note the low density of red cells on the film (profound anemia), high frequency of microspherocytes (hyperchromatic), and the large red cells (putative reticulocytes). Note the two nucleated RBCs and the Howell-Jolly body (nuclear remnant) in the macrocyte. Nucleated RBCs and Howell-Jolly bodies may be seen in autoimmune hemolytic anemia with severe hemolysis or after splenectomy. **C.** Blood film. Autoimmune hemolytic anemia. Severe. Monocyte engulfing two red cells (erythrophagocytosis). Note frequent microspherocytes and scant red cell density. **D.** Reticulocyte preparation. Autoimmune hemolytic anemia. Note high frequency of reticulocytes, the large cells with precipitated ribosomes. Remaining cells are microspherocytes. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

Serologic Features (Table 22–2)

- The diagnosis of AHA requires demonstration of immunoglobulin and/or complement bound to the RBCs.
- This is achieved by the direct antiglobulin test (DAT) in which rabbit antiserum to human IgG or complement is added to suspensions of washed patient's RBCs. Agglutination of the RBCs signifies the presence of surface IgG or complement.
- The DAT is first performed with broad-spectrum reagents, including antibodies against both complement and immunoglobulin. If this is positive, further testing is done to define the offending antibody or complement component.
- RBC may be coated with:
  — IgG alone
  — IgG and complement
  — Complement only
- Rarely, anti-IgA and anti-IgM reactions are encountered.
- Autoantibody exists in a dynamic equilibrium between RBCs and plasma.
- Free autoantibody may be detected by the indirect antiglobulin test (IAT), in which the patient's serum is incubated with normal donor RBCs, which are then tested for agglutination

by the addition of antiglobulin serum.

- Binding affinity for antibodies varies, but in general, serum autoantibody is detectable in those with heavily coated RBCs.
- A positive IAT with a negative DAT probably does not indicate autoimmune disease but an alloantibody generated by a prior transfusion or pregnancy.
- Occasional patients exhibit all the features of AHA but have a negative DAT. The amount of their RBC-bound autoantibody is too low for detection by DAT but can often be demonstrated by more sensitive methods, such as enzyme-linked immunoassay or radioimmunoassay.
- The relationship between the amount of bound antibody and degree of hemolysis is variable.
- Subclasses IgG_1 and IgG_3 are generally more effective in causing hemolysis than IgG_2 and IgG_4, apparently because of greater affinity of macrophage Fc receptors for these subclasses as well as increased complement fixation abilities.
- Autoantibodies from AHA patients usually bind to all the types of RBCs used for laboratory screening and therefore appear to be "nonspecific."
- However, the autoantibodies from individual patients usually react with antigens that are present on nearly all RBC types, the so-called "public" antigens, and only appear to lack specificity.
- Nearly half of the antibodies have specificity for epitopes on Rh proteins (Rh related) and hence will not react with cells of the rare Rh-null type.
- The remaining autoantibodies have a variety of specificities, but many are not defined.

DIFFERENTIAL DIAGNOSIS

- Other conditions may be marked by spherocytosis, including hereditary spherocytosis, Zieve syndrome, Wilson disease, and clostridial sepsis. DAT is negative in these conditions.
- AHA and autoimmune thrombocytopenia may also occur as a manifestation of systemic lupus erythematosus (secondary AHA).
- Paroxysmal nocturnal hemoglobinuria and microangiopathic hemolytic anemia should also be considered, but minimal or no spherocytosis is seen and the DAT is negative.
- If the DAT is positive for complement alone, further serologic characterizations are warranted to distinguish cold-reacting from warm-reacting autoantibodies.
- In recently transfused patients, alloantibody against donor RBCs may be detected by a positive DAT.
- Organ transplant recipients may develop a picture of AHA usually when an organ from a blood group O donor is transplanted into a group A recipient, probably because B lymphocytes persist in the transplanted organ and form alloantibodies against host RBCs.
- Marrow transplant patients of blood group O who receive blood group A or B marrow may develop a briefly positive DAT, and RBC synthesized by the engrafted marrow may be hemolyzed until previously made recipient anti-A or anti-B disappears.
- Mixed chimera also occurs so that the immunocompetent host B lymphocyte continues to generate alloantibodies.

THERAPY

- Occasional patients have a positive DAT but minimal hemolysis and stable hematocrit. These patients need no treatment but should be observed for possible progression of the disease.

Transfusion

- Generally, anemia develops slowly so that RBC transfusion is not required; however, for rapid hemolysis or patients otherwise compromised (ie, cardiac disease), transfusion may be lifesaving.
- Virtually all units are incompatible on cross-match unless one has an autoantibody that is specific for a single RBC antigen, and RBC units lacking that antigen can be obtained.
- Transfused RBCs are destroyed as fast as or faster than host RBCs but may tide the patient through a dangerous time.
- The blood bank should try to ascertain the ABO type of a patient's RBCs to avoid alloantibody-mediated hemolysis of donor cells.

Glucocorticoids

- Glucocorticoids slow or stop hemolysis in two thirds of patients.
- Twenty percent of patients will achieve a complete remission.
- Ten percent will show little or no response.
- Best results are seen in patients with primary AHA or AHA secondary to lupus erythematosus.
- Initial treatment should be with oral prednisone at 60 to 100 mg/d, orally, in adults (ie, 1.0–1.5 mg/kg body weight).
- For the gravely ill, intravenous methylprednisolone at 100 to 200 mg in divided doses over the first 24 hours can be given.
- When the hematocrit stabilizes, prednisone may be slowly tapered to 15 to 20 mg/d at a rate of about 5 mg/wk and continued for 2 to 3 months before slowly tapering off the drug entirely, if possible. In some cases in which tapering cannot be completed, alternate-day therapy may be tried, 20 to 30 mg every other day by mouth.
- Relapses are common, and the patient should be closely monitored.
- The mechanism(s) of action of glucocorticoids in AHA has not been fully established but presumably they impair macrophage ingestion of antibody-coated RBCs, early after treatment is started and may suppress autoantibody production, later.

Splenectomy

- In patients who cannot be tapered off prednisone (approximately one third), splenectomy is the next modality of therapy to consider. If response is slow and the anemia is severe, splenectomy should be considered.
- Splenectomy removes the main site of RBC destruction. Hemolysis can continue, but much higher levels of RBC-bound antibody are necessary to cause the same rate of destruction. Sometimes the amount of cell-bound antibody will decrease after splenectomy, but often no change is noted.
- Approximately two thirds of patients have complete or partial remission after splenectomy, but

relapses frequently occur. If glucocorticoids are still necessary, it is often possible to use a lower dosage.

- Splenectomy slightly increases the risk of sepsis (children more than adults), and pneumococcal, *Haemophilus influenzae* B, and meningococcal vaccine should be given several weeks before surgery, if feasible. In addition, prophylactic oral penicillin is often given to children after splenectomy.
- Asplenic patients should carry a medical alert indicator to warn about the development of sudden, severe infection.

Rituximab

- A monoclonal antibody directed against CD20 may be used to treat AHA based on its ability to eliminate B lymphocytes producing autoantibodies to RBCs. The rapid response in many patients in whom autoantibody is still circulating makes that an unlikely initial mechanism.
- Opsonized B lymphocytes may decoy macrophages and monocytes from autoantibody complexes and normalize autoreactive T lymphocyte responses.
- The response rate has averaged approximately 65% of patients treated with anti-CD20 at a dose of 375 mg/m^2 weekly for 2 to 4 weeks.

Rituximab and Glucocorticoids

- The results of combining rituximab and glucocorticoids have been ambiguous. Some studies have shown a better response using combination therapy than either drug alone and others have not.
- However, the duration of response has been shown to be longer in those individuals treated with both agents simultaneously.

Immunosuppressive Drugs

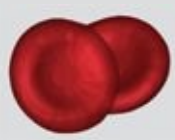
- Either cyclophosphamide (60 mg/m^2) or azathioprine (80 mg/m^2) given daily, orally, can be used.
- Close attention to blood counts is crucial because erythropoiesis can be suppressed, temporarily worsening the anemia.
- Treatment can be continued for up to 6 months awaiting a response and then tapered if and when the desired response is attained.

Other Treatments

- Improvement in the hemolytic anemia has been reported in patients with colitis after colectomy or after removal of an ovarian dermoid cyst.
- Plasmapheresis has been used with occasional success reported, but its efficacy is unpredictable.
- Variable success has been achieved with high-dose intravenous immunoglobulin (400 mg/kg daily for 5 days), danazol, cladrabine, and other uncommonly used approaches described in *Williams Hematology*, 9th ed, Chap 54.

COURSE AND PROGNOSIS

- Idiopathic warm-antibody AHA runs an unpredictable course characterized by remissions and relapses.
- Survival at 10 years is approximately 70%.
- In addition to anemia, deep venous thrombosis, pulmonary emboli, splenic infarcts, and other cardiovascular events occur during active hemolytic disease.
- In secondary warm-antibody AHA, prognosis is related to the underlying disease.
- Overall mortality rate in children is lower than in adults, ranging from 10% to 30%.
- AHA related to infection is self-limited and responds well to glucocorticoids.
- Children who develop chronic AHA tend to be older.



For a more detailed discussion, see Charles H. Packman: Hemolytic Anemia Resulting from Immune Injury, Chap. 54 in *Williams Hematology*, 9th ed.

CHAPTER 23
Cryopathic Hemolytic Anemia

- This type of anemia is caused by autoantibodies that bind red cells best at temperatures below 37°C, usually below 31°C.
- It is mediated through two major types of "cold antibody": cold agglutinins and Donath-Landsteiner antibodies.
- Clinical features vary considerably, but in both types, the complement system plays a major role in red cell destruction.

COLD AGGLUTININ-MEDIATED AUTOIMMUNE HEMOLYTIC ANEMIA

- Cold agglutinins are immunoglobulin M (IgM) autoantibodies that agglutinate red cells, optimally between 0°C and 5°C. Complement fixation occurs at higher temperatures.
- This hemolytic anemia is classified as either primary (chronic cold agglutinin disease) or secondary (generally as a result of *Mycoplasma pneumoniae* infection or Epstein-Barr virus [EBV]–related infectious mononucleosis) (Table 23–1).
- Peak incidence for the primary (chronic) syndrome is in persons older than 50 years.
- This disorder characteristically has monoclonal IgM cold agglutinins and may be considered a symptomatic monoclonal gammopathy.
- Some patients develop a B-cell lymphoproliferative disorder (eg, Waldenström macroglobulinemia).

TABLE 23–1 AUTOIMMUNE HEMOLYTIC ANEMIA: COLD ANTIBODY TYPE*

I. Mediated by cold agglutinins
  A. Idiopathic (primary) chronic cold-agglutinin disease (usually associated with clonal B-lymphocyte disease)
  B. Secondary cold-agglutinin hemolytic anemia
    1. Postinfectious (eg, *Mycoplasma pneumoniae* or infectious mononucleosis)
    2. Associated with preexisting malignant B-cell lymphoproliferative disorder
II. Mediated by cold hemolysins
  A. Idiopathic (primary) paroxysmal cold hemoglobinuria—very rare
  B. Secondary
    1. Donath-Landsteiner hemolytic anemia, usually associated with an acute viral syndrome in children—relatively common
    2. Congenital or tertiary syphilis in adults—very rare

*Uncommonly, cases may have mixed cold and warm autoantibodies (eg, primary or idiopathic mixed autoimmune hemolytic anemia) or secondary mixed autoimmune hemolytic anemia associated with the rheumatic disorders, particularly systemic lupus erythematosus.

Source: *Williams Hematology*, 9th ed, Chap. 54, Table 54–1.

Pathogenesis

- The specificity of cold agglutinins is usually against I/i antigens. I is expressed heavily in adult red cells, weakly on neonatal red cells. The reverse is true of the i antigen, which also may still be expressed on reticulocytes.
- High proportions of IgM cold agglutinins with either anti-I or anti-i specificity have heavy-chain variable regions encoded by V_H4–34, a conserved immunoglobulin variable region gene.
- Naturally occurring cold agglutinins are present in low titer (less than 1:32) in normal persons. Transient hyperproduction of less clonally restricted antibodies occurs in the recovery phase of infections, such as EBV, mycoplasma, or cytomegalovirus.
- I/i antigens serve as mycoplasma receptors, which may lead to altered antigen presentation and to subsequent autoantibody production.
- In B-cell lymphomas, cold agglutinins may be produced by the malignant lymphocytes.
- The highest temperature at which antibodies can cause red cell agglutination is termed the thermal amplitude. The higher the thermal amplitude, the greater the risk of clinically significant hemolysis, depending on the ambient temperature.
- Cold agglutinins bind to red cells in the superficial dermal vessels, where temperatures may be less than 37°C, impeding capillary flow, producing acrocyanosis.
- Hemolysis is dependent on the antibody's ability to bind complement to the red cell membrane (complement fixation); concurrent agglutination is not required for this process.
- Red cell injury then occurs either by direct lysis or enhanced phagocytosis by macrophages.
- Direct lysis results from propagation of the full complement sequence, but severe intravascular hemolysis from this cause is rare.
- Commonly, fragments C3b and C4b are deposited on the red cell surface, providing a stimulus for phagocytosis. The affected red cell may be engulfed and destroyed or released back into circulation as a spherocyte because of loss of some plasma membrane (partial phagocytosis).
- Red cells are released with a coating of C3dg, an inactive fragment that protects the red cells from further complement fixation and agglutination but results in a positive direct antiglobulin test.

Clinical Features

- Cold-agglutinin–mediated hemolysis accounts for 10% to 20% of all cases of autoimmune hemolytic anemia.
- Women are affected more commonly than men.
- Hemolysis is generally chronic, although episodes of acute hemolysis can occur on chilling.
- Acrocyanosis is frequently observed, but skin ulceration and necrosis are uncommon.
- Splenomegaly may occasionally be seen in the idiopathic form.
- The hemolysis caused by mycoplasma infection develops as the patient recovers from the infection and is self-limited, lasting 1 to 3 weeks.
- In patients with mycoplasma infections, clinically significant hemolysis is uncommon.

Laboratory Features

- Anemia is usually mild to moderate. On the blood film the red cells show **autoagglutination (Figure 23–1), polychromasia,** and **spherocytosis.**

- In the chronic syndrome, serum titers of cold agglutinins (generally IgM) can be greater than 1:100,000. The direct antiglobulin test is positive with anticomplement reagents. The cold agglutinin itself (IgM) is not detectable because it readily dissociates from the red cell at 37°C.
- As a rule, the higher the cold agglutinin titer, the higher the thermal amplitude. However, there are exceptions to this rule (lower titer and high thermal amplitude).
- Testing for cold agglutinin titer and thermal amplitude requires blood collection and serum separation at 37°C.
- Anti-I specificity is seen with idiopathic disease, *M. pneumoniae*, and some cases associated with lymphoma. Anti-i occurs with infectious mononucleosis and lymphomas. Rarely, the antibodies have other specificities, including Pr, M, or P antigens.

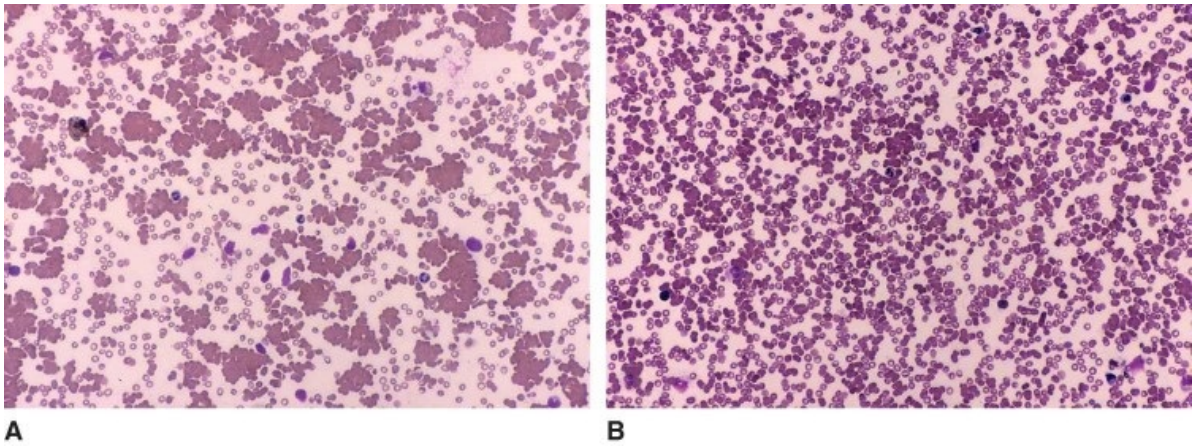


FIGURE 23–1 Blood films. **A.** Cold-reactive (IgM) antibody. Red cell agglutination at room temperature. **B.** Same blood examined at 37°C. Note marked reduction in agglutination. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

Differential Diagnosis

- When peripheral vaso-occlusive symptoms occur, especially if related to cold temperatures (Raynaud phenomenon), cryoglobulinemia should also be considered.
- In drug-induced immune hemolytic anemia, the direct antiglobulin test also may be positive only for complement.
- Mixed type autoimmune hemolysis can occur with a direct antiglobulin test positive for both IgG and complement, along with elevated cold agglutinin titers.
- Episodic hemolysis can result from paroxysmal cold hemoglobinuria (see below), paroxysmal nocturnal hemoglobinuria (see Chap. 43), march hemoglobinuria (see Chap. 19), and some red cell enzyme disorders (see Chap. 14).

Therapy, Course, and Prognosis

- Keeping the patient warm is important and may be the only treatment needed for mild conditions.
- Rituximab can be useful in symptomatic cases. Patients have responded to doses ranging from 100 to 375 mg/m^2 weekly for up to 4 weeks.
- The combination of rituximab and fludarabine has also been used successfully.
- Chlorambucil or cyclophosphamide have been used for more severe chronic cases.

- Splenectomy and glucocorticoids generally are not helpful (the latter may have some efficacy in low titer, high thermal amplitude cases), although very high dose glucocorticoids may be useful in severely ill patients.
- In critically ill patients, plasmapheresis may provide temporary relief.
- Generally, patients with the chronic syndrome have a stable condition and long-term survival.
- Postinfectious syndromes are self-limited, resolving in a few weeks.
- In patients with an associated lymphoma, treatment is usually directed at that disease.

PAROXYSMAL COLD HEMOGLOBINURIA

- This very rare form of hemolytic anemia is characterized by recurrent massive hemolysis following exposure to cold. Formerly, this condition was more common, because of its association with syphilis. A self-limited form occurs in children following several types of viral infections.

Pathogenesis

- In the extremities, the cold reactive autoantibody (Donath-Landsteiner antibody), which is an IgG antibody, and early complement proteins bind to the red cells at low temperatures. On return to the 37°C environment, lysis occurs as a consequence of propagation of the classic complement sequence.

Clinical Features

- Two percent to 5% of all patients have autoimmune hemolytic anemia; the incidence of hemolytic anemia may exceed 30% in the pediatric population.
- Paroxysms of hemolysis occur with associated systemic symptoms—rigors, fever, diffuse myalgias, and headache. These symptoms and hemoglobinuria usually last several hours. Cold-induced urticaria may also occur.

Laboratory Features

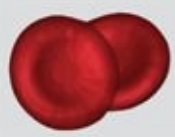
- Hemoglobinuria with a rapid fall in hemoglobin level is usual and is associated with depressed complement levels. Spherocytes and erythrophagocytosis may be seen on the blood film.
- The direct antiglobulin test is positive for complement red blood cell coating during and immediately after an attack; the Donath-Landsteiner antibody itself is not detected by the test because it readily dissociates from the red cells.
- Antibody is detected by the biphasic Donath-Landsteiner test. Red cells are incubated with the patient's serum at 4°C, then warmed to 37°C, at which point intense hemolysis occurs.
- Classically, the antibody (IgG type) has specificity for P blood group antigens, although other specificities have been noted.
- The Donath-Landsteiner antibody is a far more potent hemolysin than most cold agglutinins.

Differential Diagnosis

- Patients with paroxysmal cold hemoglobinuria lack elevated titers of cold agglutinins, distinguishing it from cold agglutinin disease.

Therapy, Course, and Prognosis

- Attacks can be prevented by avoiding cold exposure.
- Splenectomy and glucocorticoids are not of value.
- Urticaria may be treated with antihistamines.
- If related to syphilis, the hemolysis will resolve with antibiotic treatment of the infection.
- Postinfectious paroxysmal cold hemoglobinuria may resolve spontaneously in days to weeks, although the antibody may be detectable for years.
- In the idiopathic chronic form, long-term survival is common.
- Children may have a high mortality rate.



For a more detailed discussion, see Charles H. Packman: Hemolytic Anemia Resulting from Immune Injury, Chap. 54 in *Williams Hematology*, 9th ed.

CHAPTER 24

Drug-Induced Hemolytic Anemia

ETIOLOGY AND PATHOGENESIS

- Three mechanisms of drug-related immunologic injury to red cells are defined:
  — Hapten/drug adsorption involving drug-dependent antibodies
  — Ternary complex formation involving drug-dependent antibodies
  — Induction of autoantibodies that react with red cells in the absence of the inciting drug
- Drug-related nonimmunologic protein adsorption may also result in a positive direct antiglobulin test without red cell injury.
- **Table 24–1** lists the drugs implicated in the production of a positive direct antiglobulin test and accelerated red cell destruction.
- **Table 24–2** summarizes the four mechanisms of drug-induced immune interaction with the red cell surface.

TABLE 24–1 ASSOCIATION BETWEEN DRUGS AND POSITIVE DIRECT ANTIGLOBULIN TESTS*

Drugs	
Hapten or Drug Adsorption Mechanism	
Penicillins	Carbromal
Cephalosporins	Tolbutamide
Tetracycline	Cianidanol
6-Mercaptopurine	Hydrocortisone
	Oxaliplatin
Ternary Complex Mechanism	
Stibophen	Probenecid
Quinine	Nomifensine
Quinidine	Cephalosporins
Chlorpropamide	Diethylstilbestrol
Rifampicin	Amphotericin B
Antazoline	Doxepin
Thiopental	Diclofenac
Tolmetin	Etodolac
Metformin	Hydrocortisone
	Oxaliplatin
	Pemetrexed
Autoantibody Mechanism	

Cephalosporins	Cianidanol
Tolmetin	Latamoxef
Nomifensine	Glafenine
α-Methyldopa	Procainamide
l-Dopa	Diclofenac
Mefenamic acid	Pentostatin
Teniposide	Fludarabine
Oxaliplatin	Cladribine
Efalizumab	Lenalidomide
Nonimmunologic Protein Adsorption	
Cephalosporins	Cisplatin
Oxaliplatin	Carboplatin
Uncertain Mechanism of Immune Injury	
Mesantoin	Streptomycin
Phenacetin	Ibuprofen
Insecticides	Triamterene
Chlorpromazine	Erythromycin
Melphalan	5-Fluorouracil
Isoniazid	Nalidixic acid
p-Aminosalicylic acid	Sulindac
Acetaminophen	Omeprazole
Thiazides	Temafloxacin
Efavirenz	Carboplatin

*It is not always possible to infer the mechanism of immune injury induced by a drug. Moreover, some drugs can act by more than one mechanism. In cases of uncertain mechanism, the cited drug use is coincident with the hemolytic anemia, and causality is inferred, not established experimentally. These cases are included so that the reader may be aware of these potential associations.
Source: *Williams Hematology*, 9th ed, Chap. 54, Table 54–2.

TABLE 24–2 MAJOR MECHANISMS OF DRUG-RELATED HEMOLYTIC ANEMIA AND POSITIVE DIRECT ANTIGLOBULIN TESTS

	Hapten/Drug Adsorption	Ternary Complex Formation	Autoantibody Binding	Nonimmunologic Protein Adsorption
Prototype drug	Penicillin	Quinidine	α-Methyldopa	Cephalothin
Role of drug	Binds to red cell membrane	Forms ternary complex with antibody and red cell membrane component	Induces formation of antibody to native red cell antigen	Possibly alters red cell membrane
Drug affinity to cell	Strong	Weak	None demonstrated to intact red cell but binding to membranes reported	Strong
Antibody to drug	Present	Present	Absent	Absent
Antibody class predominating	IgG	IgM or IgG	IgG	None

Proteins detected by direct antiglobulin test	IgG, rarely complement	Complement	IgG, rarely complement	Multiple plasma proteins
Dose of drug associated with positive antiglobulin test	High	Low	High	High
Presence of drug required for indirect antiglobulin test	Yes (coating test red cells)	Yes (added to test medium)	No	Yes (added to test medium)
Mechanism of red cell destruction	Splenic sequestration of IgG-coated red cells	Direct lysis by complement plus splenic–hepatic clearance of C3b-coated red cells	Splenic sequestration	None

Source: *Williams Hematology*, 9th ed, Chap. 54, Table 54–3.

HAPTEN OR DRUG ADSORPTION MECHANISM

- This occurs with drugs that bind firmly to red cell membrane proteins. Penicillin is the classic example.
- In patients receiving high-dose penicillin, red cells have a substantial coating of the drug. In a small proportion of patients, an antipenicillin antibody (usually immunoglobulin G [IgG]) develops and binds to the penicillin on the red cell. Hemolytic anemia may ensue and the direct antiglobulin test becomes positive.
- Hemolytic anemia caused by penicillin typically occurs after 7 to 10 days of treatment and ceases a few days to 2 weeks after the drug is stopped.
- Other manifestations of penicillin allergy are usually not present.
- Antibody-coated ("opsonized") red cells are destroyed mainly in the spleen.
- Antibodies eluted from red cells, or present in sera, react only against penicillin-coated red cells. This specificity distinguishes drug-dependent antibodies from true autoantibodies.
- Hemolytic anemia similar to that seen with penicillin has also been ascribed to other drugs (see **Table 24–1**).

TERNARY COMPLEX MECHANISM: DRUG–ANTIBODY TARGET-CELL COMPLEX

- The mechanism of red cell injury is not clearly defined, but it appears to be mediated by a cooperative interaction to generate a *ternary complex* involving the drug or drug-metabolite, a drug-binding membrane site on the target cell, and antibody, with consequent activation of complement (**Figure 24–1B**).
- The antibody attaches to a neoantigen consisting of loosely bound drug and red cell antigen; binding of drug to the target cell is weak until stabilized by the attachment of the antibody to both drug and cell membrane.
- Some of these antibodies have specificity for blood group antigens, such as Rh, Kell, or Kidd, and are nonreactive with red cells lacking the alloantigen even in the presence of drug.

- The direct antiglobulin test is usually positive with anticomplement reagents.
- Intravascular hemolysis may occur after activation of complement, with hemoglobinemia and hemoglobinuria, and C3b-coated red cells may be destroyed by the spleen and liver.

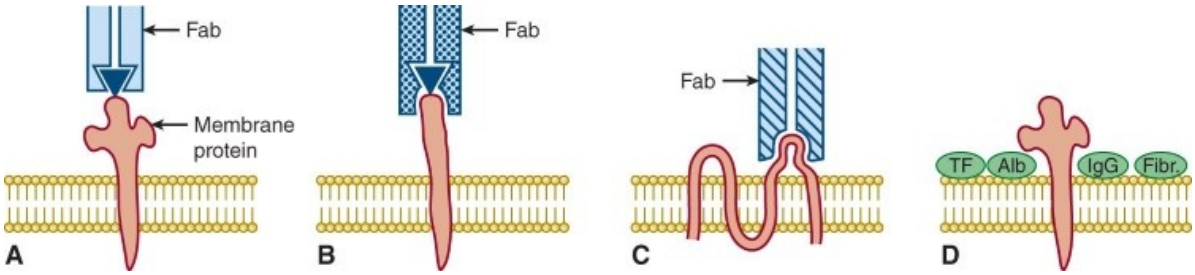


FIGURE 24–1 Effector mechanisms by which drugs mediate a positive direct antiglobulin test. Relationships of drug, antibody-combining site, and red cell membrane protein are shown. Panels A, B, and C show only a single immunoglobulin Fab region (bearing one combining site). **A.** Drug adsorption/hapten mechanism. The drug (▼) binds avidly to a red cell membrane protein in vivo. Antidrug antibody (usually IgG) binds to the protein-bound drug. The direct antiglobulin test (with anti-IgG) detects IgG antidrug antibody on the patient's circulating (drug-coated) red cells. **B.** Ternary complex mechanism. Drug binds loosely or in undetectable amounts to red cell membrane. However, in the presence of appropriate antidrug antibody, a stable trimolecular (ternary) complex is formed by drug, red cell membrane protein, and antibody. In this mechanism, the direct antiglobulin test typically detects only red cell–bound complement components (eg, C3 fragments) that are bound covalently and in large number to the patient's red cells in vivo. The antibody itself escapes detection. **C.** Autoantibody induction. Some drug-induced antibodies can bind avidly to red cell membrane proteins (usually Rh proteins) in the absence of the inducing drug and are indistinguishable from the autoantibodies of patients with autoimmune hemolytic anemia. The direct antiglobulin test detects the IgG antibody on the patient's red cells. **D.** Drug-induced non-immunologic protein adsorption. Certain drugs cause plasma proteins to attach nonspecifically to the red cell membrane. The direct antiglobulin test detects nonspecifically bound IgG and complement components. In contrast to the other mechanisms of drug-induced red cell injury, this mechanism does not shorten red cell survival in vivo. (Source: *Williams Hematology*, 9th ed, Chap. 54, Fig. 54–1.)

AUTOANTIBODY MECHANISM

- Many drugs induce the formation of autoantibodies to autologous (or homologous) red cells, most importantly α-methyldopa (see **Table 24–1**). The mechanism by which a drug can induce formation of an autoantibody is unknown.
- Positive direct antiglobulin tests are seen in 8% to 36% of those taking α-methyldopa. The positive test develops 3 to 6 months after the start of therapy. In contrast, less than 1% of those taking α-methyldopa develop hemolytic anemia.
- Infrequently, patients with chronic lymphocytic leukemia treated with purine analogs (eg, fludarabine) develop autoimmune hemolytic anemia.
- Antibodies in the serum or eluted from red cells react optimally at 37°C with autologous or homologous red cells in the absence of drug.
- As in autoimmune hemolytic anemia, these antibodies frequently react with the Rh complex.
- Destruction of red cells occurs chiefly by splenic sequestration of IgG-coated red cells.

NONIMMUNOLOGIC PROTEIN ADSORPTION

- Patients receiving cephalosporins occasionally develop positive direct antiglobulin tests as a consequence of nonspecific adsorption of immunoglobulins, complement, albumin, fibrinogen, and other plasma proteins to red cell membranes (see **Figure 24–1D**).
- Hemolytic anemia has not been reported.

- The clinical importance is the potential to complicate cross-matching.

CLINICAL FEATURES

- A careful drug history should be obtained in all patients with hemolytic anemia and/or positive direct antiglobulin test.
- The severity of symptoms depends on the rate of hemolysis, and the clinical picture is quite variable.
- Patients with hapten/drug adsorption (eg, penicillin) and autoimmune (eg, α-methyldopa) mechanisms generally exhibit mild to moderate red cell destruction with insidious onset of symptoms over days to weeks.
- If the ternary complex mechanism is operative (eg, cephalosporins or quinidine), there may be sudden onset of severe hemolysis with hemoglobinuria and acute renal failure.
- Hemolysis can occur after only one dose of the drug if the patient has been previously exposed.

LABORATORY FEATURES

- Findings are similar to those of autoimmune hemolytic anemia, with anemia, reticulocytosis, and high mean cell volume.
- Leukopenia, thrombocytopenia, hemoglobinemia, or hemoglobinuria may be observed in cases of ternary complex-mediated hemolysis.
- The serologic features are included under “Differential Diagnosis.”

DIFFERENTIAL DIAGNOSIS

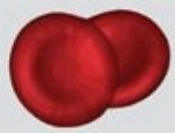
- Immune hemolysis caused by drugs should be distinguished from autoimmune hemolytic anemia (warm or cold antibodies), congenital hemolytic anemias (eg, hereditary spherocytosis), and drug-mediated hemolysis caused by disorders of red cell metabolism (eg, glucose-6-phosphate dehydrogenase deficiency).
- In drug-related hemolytic anemia, the direct antiglobulin test is positive.
- In the hapten/drug mechanism, the key difference from autoimmune hemolytic anemia is that serum antibodies react only with drug-coated red cells. This serologic distinction plus a history of the specific drug exposure should be decisive.
- In the ternary complex mechanism, the direct antiglobulin test is positive with anticomplement serum, similar to cold autoimmune hemolytic anemia. However, the cold agglutinin titer and Donath-Landsteiner test are normal, and the indirect antiglobulin test is positive only in the presence of drug. The direct antiglobulin test becomes negative shortly after stopping the drug.
- In hemolytic anemia caused by a drug in the α-methyldopa category, the direct antiglobulin reaction is strongly positive for IgG (rarely for complement) and the indirect antiglobulin reaction is positive with unmodified red cells, often showing Rh specificity. There is no specific serologic test to differentiate this disorder from warm-autoimmune hemolytic anemia with Rh complex specificities. The diagnosis is supported by recovery from anemia and

disappearance of antibodies on discontinuing the drug.

- With a clinical picture of drug-induced immune hemolysis, it is reasonable to stop any drug while serologic studies are performed and to monitor for decrease in reticulocytosis, increase in hematocrit, and disappearance of positive antiglobulin test.
- Rechallenge with the suspected drug may confirm the diagnosis but should be tried only for compelling reasons.

THERAPY, COURSE, AND PROGNOSIS

- Discontinuation of the offending drug is often the only treatment needed and may be lifesaving in severe hemolysis mediated by the ternary complex mechanism.
- Transfuse only for severe, life-threatening anemia.
- Glucocorticoids are generally unnecessary and are of questionable efficacy.
- If high-dose penicillin is the treatment of choice in life-threatening infection, therapy need not be changed as a result of a positive direct antiglobulin test unless there is overt hemolytic anemia.
- A positive direct antiglobulin test alone is not necessarily an indication for stopping a drug in the α-methyldopa category, although it may be prudent to consider alternative therapy.
- Hemolysis associated with α-methyldopa type drug ceases promptly after stopping the drug. The positive direct antiglobulin test gradually diminishes over weeks or months.
- Problems with cross-matching may occur in patients with a strongly positive indirect antiglobulin test.
- Immune hemolysis caused by drugs is usually mild, but occasional episodes of severe hemolysis with renal failure or death have been reported, usually as a consequence of the ternary complex mechanism.



For a more detailed discussion, see Charles H. Packman: Hemolytic Anemia Resulting from Immune Injury, Chap. 54 in *Williams Hematology*, 9th ed.

CHAPTER 25

Alloimmune Hemolytic Disease of the Newborn

DEFINITION

- In this disease, there is fetal to maternal transfer of red cells that results in immunization of the mother. Then, transplacental transfer of maternal anti–red cell antibodies to the fetus shortens the life span of fetal or newborn red cells.
- Manifestations include fetal hemolytic anemia, jaundice, and hepatosplenomegaly; in more severe cases, anasarca and kernicterus also occur.

PATHOGENESIS

- Asymptomatic transplacental passage of fetal red cells occurs in 75% of pregnancies.
- If there is blood group incompatibility between mother and fetus, the chance of maternal immunization increases with the volume of any transplacental hemorrhage.
- Approximately 95% of pregnant women have fetomaternal hemorrhage of less than 1.0 mL at delivery.
- Intrapartum fetomaternal hemorrhage of more than 30 mL occurs in approximately 1.0% of deliveries.
- Larger volume transplacental hemorrhages are more likely to occur at delivery or during invasive obstetric procedures.
- The risk of sensitization increases with each trimester of pregnancy and is greatest (65%) at delivery.
- Fetomaternal transfusion can occur at the time of chorionic villous sampling, amniocentesis, therapeutic abortion, cesarean section, abdominal trauma, and other situations.
- Prior blood transfusions or abortions also can immunize the mother.
- Maternal red cell antibodies fall into three classes: antibodies directed against the D antigen in the Rh blood group, antibodies directed against the A or B antigens, and antibodies directed against any of the remaining red cell antigens.
- The D antigen of the Rh blood group system is involved in most serious cases.
- Without prophylaxis, immunization occurs in approximately 12% of those at risk with an RhD-positive, ABO-compatible fetus and 2% of these with an RhD-positive, ABO-incompatible fetus.
- Anti-D immunoglobulin G (IgG) crosses the placenta and leads to a positive antiglobulin test and hemolysis in the infant.
- In ABO hemolytic disease, the mother is usually type O and the fetus is either type A or B.
- Anti-A and anti-B antibodies ordinarily cause mild, rarely severe, hemolysis. Numerous other causal antibodies have been described but are less common (see “Epidemiology”).

EPIDEMIOLOGY

- The distribution of blood group antigens among different ethnic groups determines their risk of alloimmune hemolytic disease.
- Approximately 16% of Americans of European descent are RhD-negative, compared to 8% of Americans of African ancestry, 5% of persons of Asian Indian ancestry, and 0.3% of those of Chinese ancestry.
- More than 50 different red cell antigens have been associated with maternal alloimmunization and with alloimmune hemolytic disease with varying degrees of severity.
- Women have naturally occurring antigens to blood group A or B (eg, mother type O) or may develop other antibodies not screened for prior to blood transfusion.
- Antenatal screening programs detect antibodies in approximately 0.2% of pregnant women.
- After anti-RhD, the following blood groups are most often involved in alloimmunization: Rh (C, e, E, e), Kell, Duffy, Kidd, and the MNS.
- The presence of maternal antibodies is not predictive of alloimmune hemolytic disease because: (1) they may be IgM antibodies and not traverse the placenta, (2) the antigens may not be present on fetal red cells or their density is very low, (3) the concentration of antibody in maternal blood may be very low, (4) the antibody Ig subclass may not interact with fetal red cells, and (5) other mitigating factors.

CLINICAL FEATURES

Distinctions Between ABO and RhD Alloimmunization

- RhD and ABO hemolytic disease differ in several respects (**Table 25–1**).
  - — ABO hemolytic disease can occur in (1) mothers with O red cells and fetuses with blood group A or B red cells, (2) mothers of type B and fetuses of type A, and (3) mothers of type A and fetuses of type B.
  - — ABO incompatibility is present in 15% of O group pregnancies, but hemolytic disease of the fetus or newborn occurs in about 2% of births.
  - — The low frequency of ABO hemolytic disease is the result of most anti-A and anti-B being IgM antibodies, which do not easily traverse the placenta.
  - — Prenatal testing for maternal anti-A or anti-B antibodies is not predictive of occurrence of alloimmune hemolytic disease because of the unpredictable time of expression of A or B on fetal red cells and the sink for maternal antibodies provided by other fetal tissues that express A or B antigen.
  - — ABO incompatibility may be observed during the first pregnancy because of preexisting anti-A and anti-B in the mother. This is not so in RhD alloimmunization, unless the mother was previously immunized by transfusion or, rarely, by sharing needles with an RhD-positive intravenous drug abuser.
  - — ABO alloimmune hemolytic disease usually results in early neonatal jaundice requiring phototherapy, but it only rarely requires exchange transfusion. Moderate anemia and mild hepatosplenomegaly may also be evident.
  - — ABO fetomaternal incompatibility rarely leads to severe disease (ie, hydrops fetalis).
  - — A somewhat higher degree of jaundice is seen in some ethnic groups (eg, Americans of

African, Southeast Asian, or Hispanic descent). This finding may have to do with variant glucuronyltransferase gene expression.

TABLE 25–1 COMPARISON OF RH AND ABO HEMOLYTIC DISEASE OF THE NEWBORN

	Rh	ABO
Blood groups		
Mother	Negative	O
Infant	Positive	A or B
Type of antibody	IgG_1 and/or IgG_3	IgG_2
Clinical aspects		
Occurrence in first-born	5%	40%–50%
Predictable severity in subsequent pregnancies	Usually	No
Stillbirth and/or hydrops	Frequent	Rare
Severe anemia	Frequent	Rare
Degree of jaundice	+++	+ to ++
Hepatosplenomegaly	+++	+
Laboratory findings		
Maternal antibodies	Always present	Not clear-cut
Direct antiglobulin test (infant)	+	+ or –
Microspherocytes	0	+
Treatment		
Antenatal measures	Yes	No
Exchange transfusion frequency	Approximately 2/3	Occasional
Donor blood type	Rh-negative, group specific when possible	Group O only
Incidence of late anemia	Common	Rare

Source: *Williams Hematology*, 9th ed, Chap. 55, Table 55–3.

Hemolytic Disease

- Anemia, jaundice, and hepatosplenomegaly in the newborn are the major findings in alloimmune hemolytic disease.
- The hemolytic rate can be determined by measuring using an exhaled carbon monoxide end-tidal breath analyzer. Quantification of hemolytic rate is neonates is accomplished in 5 minutes, and it is safe and noninvasive using nasal cannula.
- The spectrum of severity is wide. In RhD alloimmunization, 50% of newborns have mild disease and do not need intervention, 25% are born at term with moderate anemia and severe jaundice, and 25% of fetuses developed hydrops fetalis in utero prior to the availability of intrauterine intervention.
- With severe hemolysis, usually in RhD-sensitized mothers, profound anemia can lead to hydrops fetalis (anasarca caused by hypoproteinemia, cardiac failure), and such fetuses can die in utero (**Figure 25–1**).
- Hydrops fetalis is associated with marked extramedullary hematopoiesis in the liver, spleen, kidneys, and adrenal glands. Portal and umbilical vein hypertension, hypoproteinemia (liver dysfunction), and pleural effusions and ascites can occur.
- With milder cases, hemolysis persists until incompatible red cells or the offending IgG is

cleared (half-life of IgG is 3 weeks).

- If severe anemia is present, infant displays pallor, tachypnea, tachycardia; cardiovascular collapse and tissue hypoxia can occur if hemoglobin is less than 40 g/L.
- Most affected infants are not jaundiced at birth because of transplacental transport of bilirubin. Jaundice appears during the first postpartum day or in hours after birth if severe hemolysis is present.
- Generally, with mild disease, the bilirubin peaks at day 4 or 5 postpartum and declines slowly thereafter.
- Premature infants may have higher levels of bilirubin of longer duration because of decreased hepatic glucuronyltransferase activity.
- With marked elevation of the serum bilirubin level, kernicterus may develop from deposition of unconjugated bilirubin in the basal ganglia and brainstem nuclei.
- Acute bilirubin encephalopathy is marked initially by lethargy, poor feeding, and hypotonia. If unaddressed, may progress to high-pitched cries, fever, hypertonia, opisthotonus, and irregular respiration.
- Severe involvement can be fatal or lead to long-lasting severe neurologic defects (eg, choreoathetoid cerebral palsy, sensorineural hearing loss, gaze abnormalities, cognitive abnormalities).
- Occasionally, severe thrombocytopenia or hypoglycemia also occurs and is a poor prognostic sign.

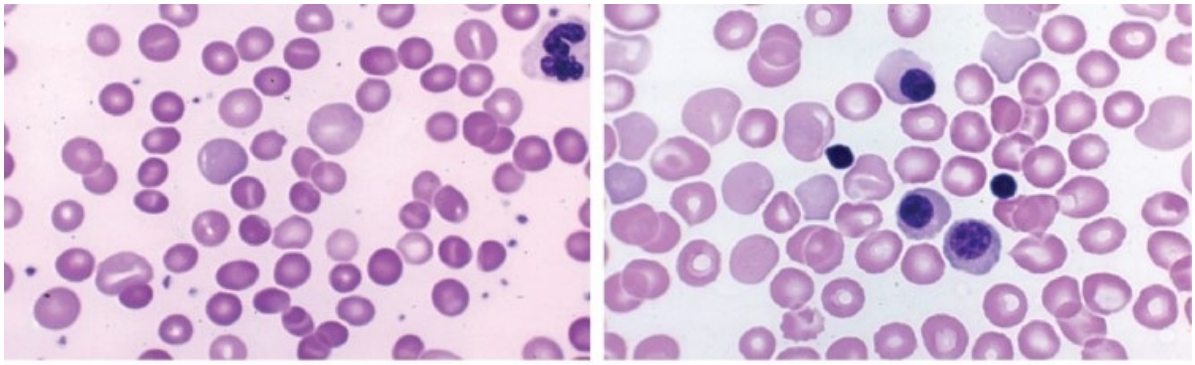


FIGURE 25–1 Alloimmune hemolytic disease of the newborn. Blood films. **A.** Infant with ABO blood group alloimmune hemolytic anemia. Note the high prevalence of spherocytes and the large polychromatophilic cells, indicative of reticulocytosis. **B.** Infant with Rh blood group alloimmune hemolytic anemia. Note spherocytes, reticulocytes, and the nucleated red cells. The intense erythroblastosis is characteristic of Rh blood group alloimmune hemolytic anemia and is less prominent in ABO blood group alloimmune hemolytic anemia. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

LABORATORY EVALUATION

Historical Guideposts

- Obstetric history often guides the laboratory approach. Prior history of transfusions, alloimmunization, severity of prior alloimmune hemolytic disease, prior hydrops fetalis (recurs in 90% of immunized mothers), neonatal death, determining paternity in subsequent pregnancies (because the fetus is at risk only if the father is positive for the antigen in question), and related factors may guide the timing and extent of fetal surveillance.

Maternal Red Cell Antigen Typing and Titering

- All pregnant patients should have ABO and RhD typing and testing for unusual red cell alloantibodies early in the pregnancy (10th to 16th week).
- Whether RhD-positive or negative, the mother should be tested again at 28 weeks gestation.
- If alloimmunized, the mother's titer should be determined at 4-week intervals from 20 to 28 weeks and every 2 weeks thereafter (**Figure 25–2**).
- Antibody titers are reported as the reciprocal of the highest dilution at which agglutination is observed. A difference of two dilutions is considered significant. If the titer becomes greater than 16 (varies from 8 to 32 in different laboratories), ultrasonography and amniocentesis can be performed to test for the bilirubin level, which predicts disease severity.
- In the United States and the United Kingdom, the anti-D level is compared to an international standard and reported in international units per milliliter (IU/mL). Levels above 4 IU/mL require prompt referral to fetomaternal specialist for monitoring and risk assessment. IU of 4 to 15/mL indicates moderate alloimmune hemolytic disease is likely and over 15 IU/mL implies a high risk of alloimmune hemolytic disease is present.
- The significance for antibody titer levels, if anti-D is not involved (eg, anti-Kell antibodies), has not been determined.

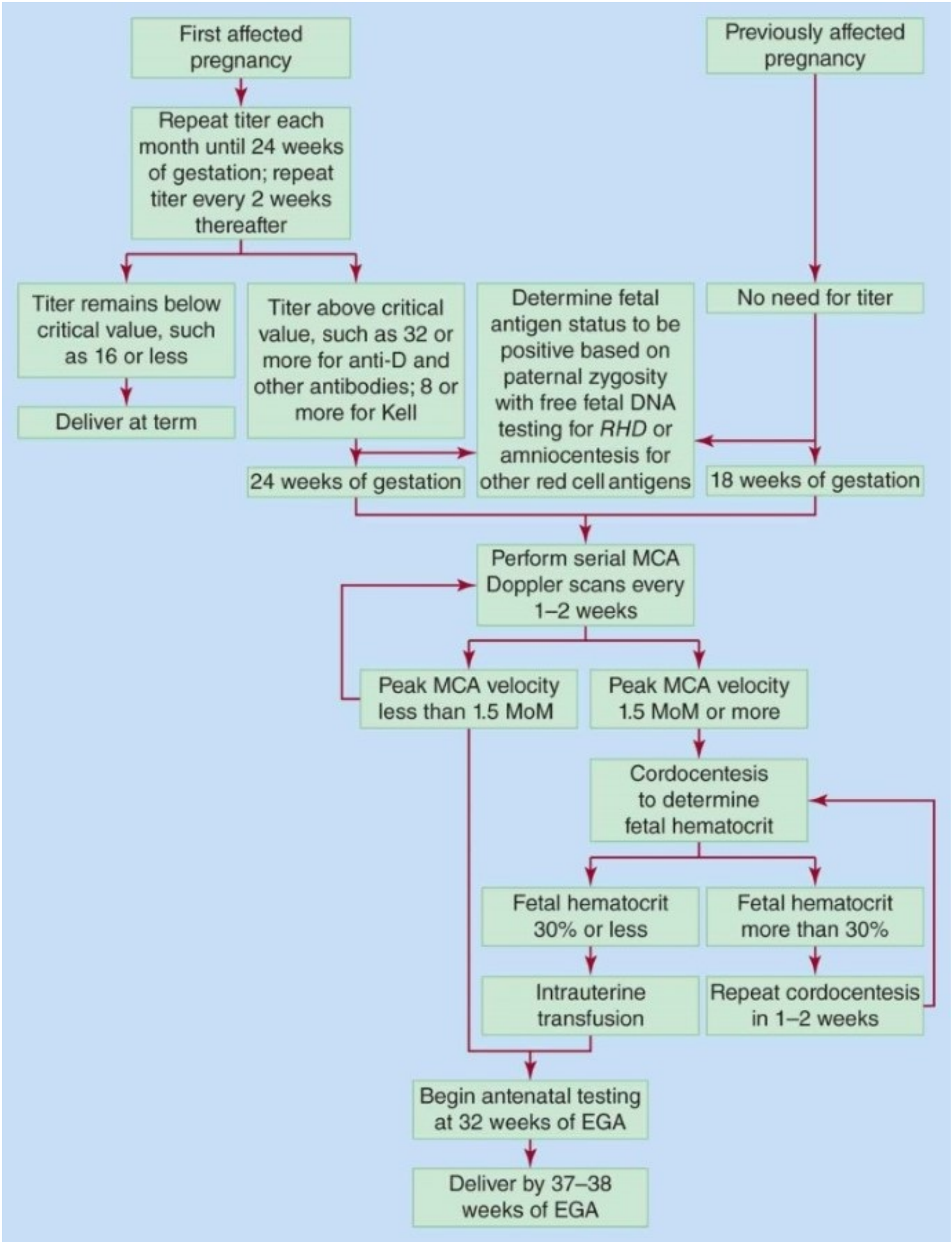

First affected pregnancy
Previously affected pregnancy
Repeat titer each month until 24 weeks of gestation; repeat titer every 2 weeks thereafter
Titer remains below critical value, such as 16 or less
Deliver at term
Titer above critical value, such as 32 or more for anti-D and other antibodies; 8 or more for Kell
Determine fetal antigen status to be positive based on paternal zygosity with free fetal DNA testing for RHD or amniocentesis for other red cell antigens
No need for titer
24 weeks of gestation
18 weeks of gestation
Perform serial MCA Doppler scans every 1–2 weeks
Peak MCA velocity less than 1.5 MoM
Peak MCA velocity 1.5 MoM or more
Cordocentesis to determine fetal hematocrit
Fetal hematocrit 30% or less
Fetal hematocrit more than 30%
Intrauterine transfusion
Repeat cordocentesis in 1–2 weeks
Begin antenatal testing at 32 weeks of EGA
Deliver by 37–38 weeks of EGA

FIGURE 25–2 Algorithm for clinical management of Rh alloimmunized pregnancy. EGA, estimated gestational age; MCA, middle cerebral artery; MoM, multiples of the median for gestational age. (Adapted with permission from Moise KJ Jr: Management of rhesus alloimmunization in pregnancy, *Obstet Gynecol.* 2008 Jul;112(1):164-176).

Paternal Zygosity Testing

- In pregnancies in which maternal sensitization occurs or there is a past history of alloimmunization, determining paternal zygosity for all common antigens involved in alloimmune hemolytic disease may determine the risk to the fetus. If the father is homozygous for the antigen in question, one can assume the fetal red cells will carry that antigen. If the father is heterozygous, the fetus will have a 50% chance of carrying the antigen.
- If paternal zygosity is unknown, testing fetal red cell blood type early in pregnancy permits appropriate monitoring of the fetus or determines that invasive fetal monitoring is unnecessary.

Fetal DNA, Amniotic Fluid Bilirubin, and Middle Cerebral Artery Doppler Measurements

- Fetal DNA can be obtained from maternal plasma in the first trimester of pregnancy (as early as 5 weeks). Real-time quantitative polymerase chain reaction methods can distinguish maternal from fetal DNA and then amplify fetal exons that include RhD. Accuracy of RhD phenotyping using fetal DNA is 95%.
- Amniotic fluid spectrophotometry for bilirubin is an indirect measure of degree of hemolysis. Bilirubin is measured at an optical density (OD) of 450 nm and the elevation in OD_{450} reflects the concentration of bilirubin derived from the fetus. A special nomogram is used to determine the bilirubin for gestational age and four zones in which the bilirubin may fall, providing the probability ranging from no risk of hemolysis to severe hemolytic anemia.
- Because of the risk of amniocentesis, this approach has been replaced by serial noninvasive middle cerebral artery Doppler ultrasound measurements for measuring fetal anemia. Measuring peak blood flow, usually at 1- to 2-week intervals after week 18 until week 35 is a more accurate assessment of anemia than amniotic fluid bilirubin levels. After week 38 of gestation, because of a high false-positive rate with Doppler measurements, amniocentesis and measurement of amniotic fluid OD_{450} is required.

Ultrasonography

- Ultrasonography allows noninvasive description of the fetal condition, an estimate of the need for aggressive management, and a biophysical profile to determine fetal well-being.
- Ultrasonography can be done serially and can detect signs of hydrops such as polyhydramnios, placental enlargement, hepatomegaly, pericardial effusion, ascites, scalp edema, and pleural effusion in roughly that sequence of appearance.

Percutaneous Umbilical Blood Sampling

- More specific information can be obtained by percutaneous umbilical blood sampling (PUBS) (mortality < 1%) or chorionic villus sampling.
- PUBS (also called cordocentesis) allows direct measurement of fetal red cell antigens, blood hemoglobin, reticulocyte count, direct antiglobulin test, bilirubin level, blood gases, and lactate levels and can be done at 18 weeks gestation, if severe fetal anemia is indicated by

amniotic fluid bilirubin levels or by middle cerebral artery Doppler peak flow measurements.

- PUBS can be done through a 22-gauge spinal needle inserted into the umbilical vein at the site of the cords insertion into the placenta under ultrasonic guidance. If necessary, red cell transfusion can be given by this route.
- Complications of PUBS include umbilical cord bleeding, chorioamnionitis, fetomaternal hemorrhage and maternal red cell sensitization, and fetal death. The latter is reported as approximately 3%.

Neonatal Assessment

- After delivery, the cord blood of the neonate should be sampled for hemoglobin and bilirubin concentrations, and it should be used to determine the ABO and Rh type. The direct antiglobulin test should also be performed. The blood film may show nucleated red blood cells, microspherocytes, and polychromatophilia, if alloimmune hemolytic anemia has occurred. Such testing with a blood group O RhD-positive mother is useful to detect ABO alloimmunization before the newborn is discharged.
- One hour after delivery, blood should be drawn from the mother to evaluate the degree of fetomaternal hemorrhage, so that an appropriate dose of anti-Rh IgG can be given (see "Therapy, Course, and Prognosis").
- Erythropoiesis may be suppressed in the newborn, but marrow recovery is usually complete by 2 months.
- Other diseases can cause hydrops (eg, alpha-thalassemia; see Chap. 15) but are distinguished from alloimmune hemolysis by the absence of maternal antibodies.

THERAPY, COURSE, AND PROGNOSIS

Fetus

- PUBS (see "Laboratory Evaluation") can be used to deliver a red cell transfusion to a severely affected fetus, based on level of anemia, development of ascites, or a rising bilirubin concentration. This approach has replaced exchange transfusion in some centers because it is a more rapid procedure.
- Packed red cells are transfused to the fetus to achieve a hematocrit of 40% to 45%. The red cells are packed to about 75% and a calculation made as to what volume of red cells should be necessary to achieve the desired hematocrit in the fetus.
- Intraperitoneal fetal transfusions may be necessary if (1) intravascular access is not possible because the umbilical vessels are too narrow in early pregnancy or (2) fetal size blocks access to the cord later in pregnancy.
- For a woman alloimmunized in a previous pregnancy, fetal transfusions should begin 10 weeks before the time of the earliest prior fetal death or transfusion, but not before 18 weeks gestation unless hydrops is present. Transfusions are given to keep the hematocrit of the fetus in the 20% to 25% range and to prevent hydrops.
- O-negative, antigen-negative for any other identified antibody, cytomegalovirus-negative, irradiated packed red cells are used, cross-matched against the mother's blood.
- The decision about when to deliver the fetus is complex; if possible, transfusions are given up

to 34 weeks with delivery at 36 weeks gestation.

- Other treatments to desensitize the mother (maternal immunomodulation) have included intravenous immunoglobulin with or without plasmapheresis, glucocorticoids, or administration of recombinant D-specific antibodies that do not destroy RhD-positive red cells. The nonhemolytic anti-D enters the fetal circulation and competes with natural, hemolytic anti-D for red cell sites, ameliorating the hemolysis.

Neonatal

- The aim of treatment is to prevent bilirubin neurotoxicity.
- Indications for immediate exchange transfusion:
  — The cord blood hemoglobin level is significantly less than normal (perhaps a threshold of ≤110 g/L).
  — The bilirubin level is greater than 4.5 mg/dL.
  — Cord blood bilirubin is rising rapidly (> 0.5 mg/dL per hour).
- If the infant is premature or has unstable vital signs, less stringent criteria are used to give an exchange transfusion. After the first exchange, the rate of rise of bilirubin is used to guide to subsequent transfusions.
- Double volume exchanges will remove, perhaps, 85% of sensitized red cells and greater than 50% of intravascular bilirubin, and also some maternal anti-D antibody.
- In some centers, prior to exchange, intravenous albumin is given to mobilize extravascular, interstitial bilirubin. Removal of sensitized red cells and prevention of bilirubin formation is the most efficient approach.
- ABO-compatible, RhD-negative, irradiated blood is used, cross-matched against the mother.
- Potential newborn complications of exchange transfusion include hypocalcemia, hypoglycemia, thrombocytopenia, dilutional coagulopathy, neutropenia, disseminated intravascular coagulation, umbilical venous or arterial thrombosis, enterocolitis, and infection. Permanent serious sequelae or neonatal death has been reported in a rate as high as 12% in sick infants compared to less than 1% in healthy infants over a period of observation from 1981 to 1995.
- Recombinant human erythropoietin, 200 U/kg, subcutaneously, three times per week for 6 weeks, has been used to enhance recovery of the hemoglobin concentration and decrease the need for postnatal exchange transfusions. It is also useful in Kell antigen-mediated alloimmune disease, because in that case, erythroid hypoplasia is an important factor.
- Phototherapy is used prophylactically in any patient with moderate or severe hemolysis or in infants with bilirubin levels rising at more than 0.5 mg/dL per hour and is the mainstay of treatment for unconjugated hyperbilirubinemia. The object is to prevent bilirubin neurotoxicity.
- Intensive phototherapy (≥30 microwatts/cm^2) in the 430–490 nm band is delivered to as much of the infants' surface area as possible.
- In full-term infants (at least 38 weeks gestation) with alloimmune hemolytic disease, intensive phototherapy should be instituted if total serum bilirubin is greater than or equal to 5.0 mg/dL at birth, greater than or equal to 10 mg/dL at 24 hours after birth, or greater than or equal to 13 mg/dL 48 to 72 hours after birth.
- Phototherapy is recommended at lower bilirubin levels for preterm or ill infants or infants with a positive direct antiglobulin test, often at serum bilirubin less than 5.0 mg/dL to lessen

the need for exchange transfusions.

- Other treatments have been applied. For example, administration of high-dose intravenous immunoglobulin as soon as possible after diagnosis of alloimmune hemolysis is made, decreases the need for phototherapy or exchange transfusion by nonspecific blockade of macrophage Fc receptors and, thereby, a decrease in hemolysis.
- Perinatal survival is greater than 90% with intrauterine transfusions in nonhydropic fetuses with severe alloimmune hemolytic disease. The overall survival for hydropic fetuses is approximately 85% despite intrauterine transfusion.
- A first-trimester screening program increased survival in Kell antigen induced alloimmune hemolytic disease from 61% to 100% in the Netherlands.

PREVENTION

- Transfusion of red cells matched for RhD, other Rh antigens, and for Kell antigens should be used in premenopausal women.
- RhIg immunoprophylaxis is standard practice for an RhD-negative mother (**Table 25–2**).
- Intramuscular doses of 100 to 300 µg of Rh immune globulin to nonsensitized RhD-negative mothers within 72 hours of delivery have decreased Rh immunization by greater than 90%.
- If the mother is RhD-negative with an RhD-positive newborn, administration of antepartum Rh immunoglobulin at 28 weeks has decreased immunization to about 0.1%. Rarely, sensitization may occur before the 28th week. This approach is standard practice in the United States.
- The standard dose of 300 µg of RhIg (1500 IU) affords protection for a fetomaternal transfusion of 15 mL of RhD-positive red cells or 30 mL or RhD-positive whole blood.
- Larger fetomaternal transfusions can occur in certain circumstances. The blood of RhD-negative women should be tested 1 hour after delivery of an RhD-positive infant. If abruptio placenta or abdominal trauma occurred, the testing can be done after 20 weeks gestation. Testing uses a rosette test requiring very small amounts of maternal blood, followed by a Kleihauer-Betke test for fetal red cells in the maternal blood. Flow cytometric methods are particular useful for quantification of fetal red cells in the maternal blood.
- In patients found to have large fetomaternal transfusions, larger doses of RhIg can be calculated to try to prevent maternal immunization.
- Although immunoprophylaxis has greatly reduced the incidence of alloimmune hemolytic disease, alloimmune sensitization still occurs in 10.6 per 10,000 births in the United States.
- Because the only adequate prophylaxis is for the D-antigen, other less common antibodies will continue to cause hemolytic disease.

TABLE 25–2 DOSAGE OF RH IMMUNOGLOBULIN

Indication	Route of Administration	Dose
Pregnancy termination < 12 weeks gestation	IM	50 µg
Abortion, miscarriage, ectopic pregnancy, or other pregnancy complications > 12 weeks gestation	IM, IV	300 µg
Amniocentesis or chorionic villus sampling < 34 weeks gestation	IM IV	300 µg[1] 300 µg

Amniocentesis, chorionic villus sampling, or other manipulation during pregnancy > 34 weeks gestation	IM	300 µg[2]
Obstetric complication (eg, abruptio placentae or placenta previa)	IM, IV	300 µg
Antepartum, 28 weeks gestation	IM, IV	300 µg
Postpartum[3]	IM IV	300 µg[4] 120 µg[4]
Transfusion of Rh-positive blood	IM	20 µg/mL RBCs

[1]To be repeated at 12-week intervals until delivery.

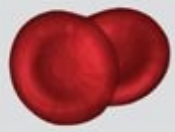
[2]Same dose should be administered if procedure is repeated 21 days after first dose.

[3]Infant should be RhD-positive.

[4]Dose should be adjusted for fetal-maternal hemorrhage > 15 mL.

Abbreviations: RBC, red blood cell; IM, intramuscular; IV, intravenous.

Reproduced with permission from Hartwell EA: Use of Rh immune globulin: ASCP practice parameter. American Society of Clinical Pathologists, *Am J Clin Pathol.* 1998 Sep;110(3):281-292.



For a more detailed discussion, see Ross M. Fasano, Jeanne E. Hendrickson, and Naomi L. C. Luban: Alloimmune Hemolytic Disease of the Fetus and Newborn, Chap. 55 in *Williams Hematology,* 9th ed.

CHAPTER 26
Hypersplenism and Hyposplenism

THE SPLEEN

- The white pulp (lymphoid tissue) functions in antigen processing and antibody production.
- The red pulp (monocyte-macrophage system) serves as a filter, retaining defective blood cells and foreign particles.

HYPERSPLENISM (INCREASED SPLENIC FUNCTION)

- Hypersplenism is considered "appropriate" if it is an exaggeration of normal function, as in hereditary spherocytosis or idiopathic thrombocytopenic purpura, or "inappropriate" if the hyperfunction is a result of vascular congestion or infiltrative disease.
- It is usually associated with splenomegaly.
- It causes cytopenias with associated compensatory bone marrow hyperplasia.
- It usually is corrected by splenectomy, if indicated.
- **Table 26–1** lists the causes of hypersplenism. **Table 26–2** lists the causes of massive splenomegaly.

TABLE 26–1 CLASSIFICATION AND THE MOST COMMON CAUSES OF SPLENOMEGALY AND HYPERSPLENISM

I. Congestive
- A. Right-sided congestive heart failure
- B. Budd-Chiari syndrome (hepatic vein thrombosis with or without inferior vena cava extension)
- C. Cirrhosis with portal hypertension
- D. Portal or splenic vein thrombosis

II. Immunologic
- A. Viral infection
  1. Acute HIV infection/chronic infection
  2. Acute mononucleosis
  3. Dengue fever
  4. Rubella (rare except newborns)
  5. Cytomegalovirus infection (rare except newborns)
  6. Herpes simplex (rare except newborns)
- B. Bacterial infection
  1. Subacute bacterial endocarditis
  2. Brucellosis
  3. Tularemia
  4. Melioidosis
  5. Listeriosis
  6. Plague
  7. Secondary syphilis
  8. Relapsing fever

9. Psittacosis
10. Anaplasmosis (formerly ehrlichiosis)
11. Rickettsial diseases (scrub typhus, Rocky Mountain spotted fever, Q fever)
12. Tuberculosis
13. Splenic abscess (most common organisms are Enterobacteriaceae, *Staphylococcus aureus*, streptococcus group D, and anaerobic organisms as part of mixed flora infections)

C. Fungal Infection
1. Blastomycosis
2. Histoplasmosis
3. Systemic candidiasis and hepatosplenic candidiasis

D. Parasitic infection
1. Malaria
2. Kala-azar
3. Leishmaniasis
4. Schistosomiasis
5. Babesiosis
6. Coccidioidomycosis
7. Paracoccidioidomycosis
8. Trypanosomiasis (cruzi, brucei)
9. Toxoplasmosis (rare except newborns)
10. Echinococcosis
11. Cysticercosis
12. Visceral larva migrans (*Toxocara* infection)

E. Inflammatory/autoimmune
1. Systemic lupus erythematosus (SLE)
2. Felty syndrome
3. Juvenile rheumatoid arthritis
4. Autoimmune lymphoproliferative syndrome (ALP syndrome)
5. Hemophagocytic syndrome
6. Common variable immunodeficiency
7. Anti-D immunoglobulin administration

III. Associated with hemolysis
A. Thalassemia major and intermedia
B. Pyruvate kinase deficiency
C. Hereditary spherocytosis
D. Autoimmune hemolytic anemia (rare)
E. Sickle cell disease, more common in early childhood (splenic sequestration), hemoglobin C disease, and some other hemoglobinopathies

IV. Infiltrative
A. Nonmalignant
1. Splenic hematoma (splenic cysts are usually a late complication of a hematoma)
2. Littoral cell angioma
3. Disorders of sphingolipid metabolism
a. Gaucher disease
b. Niemann-Pick disease
4. Cystinosis
5. Amyloidosis (light-chain amyloid and amyloid A protein)
6. Multicentric Castleman disease
7. Mastocytosis
8. Hypereosinophilic syndrome
9. Sarcoidosis

B. Extramedullary hematopoiesis
1. Primary myelofibrosis
2. Osteopetrosis (childhood)
3. Thalassemia major

C. Malignant
1. Hematologic
a. Chronic lymphocytic leukemia (especially prolymphocytic variant)
b. Chronic myeloid leukemia

c. Polycythemia vera
d. Hairy cell leukemia
e. Heavy chain disease
f. Hepatosplenic lymphoma
g. Acute leukemia (acute lymphoblastic leukemia/acute myeloid leukemia)
h. Hodgkin and other lymphomas
2. Nonhematologic
a. Metastatic carcinoma (rare)
b. Neuroblastoma
c. Wilms tumor
d. Leiomyosarcoma
e. Fibrosarcoma
f. Malignant fibrous histiocytoma
g. Kaposi sarcoma
h. Hemangiosarcoma
i. Lymphangiosarcoma
j. Hemangioendothelial sarcoma

V. Iatrogenic
A. Granulocyte colony-stimulating factor administration
B. Erythropoietin administration

Source: *Williams Hematology,* 9th ed, Chap. 56, Table 56–1.

TABLE 26–2 CAUSES OF MASSIVE SPLENOMEGALY

I. Myeloproliferative disorders
A. Primary myelofibrosis
B. Chronic myeloid leukemia

II. Lymphomas
A. Hairy cell leukemia
B. Chronic lymphocytic leukemia (especially prolymphocytic variant)

III. Infectious
A. Malaria
B. Leishmaniasis (kala azar)

IV. Extramedullary hematopoiesis
A. Thalassemia major

V. Infiltrative: Gaucher disease

Source: *Williams Hematology,* 9th ed, Chap. 56, Table 56–2.

Pathophysiology

- The normal spleen carries out filtration and elimination of aged and defective blood cells.
- This same process also removes red cells with hereditary abnormalities of red blood cell membrane and antibody-coated blood cells.
- An enlarged spleen may sequester and destroy normal blood cells, leading to symptomatic cytopenias.
- An expanded splenic (systemic) plasma pool may cause further anemia by dilution.
- Massively increased splenic blood flow, especially if there is decreased hepatic compliance, may cause portal hypertension, further splenomegaly, and associated gastroesophageal varices.
- Massive splenomegaly will cause early satiety, mediated by mechanical effects on the stomach.

Effect on Platelets

- Normally, about one third of the platelet mass is sequestered in the spleen.
- Up to 90% of platelets may be sequestered temporarily by a very enlarged spleen.
- Platelets survive almost normally in the spleen and are available, albeit slowly, when needed.

Effect on Neutrophils

- A large fraction of the circulating neutrophil pool may be marginated in an enlarged spleen.
- Neutrophils survive almost normally in the spleen and, like platelets, slowly become available on demand.

Effect on Red Blood Cells

- Red blood cells are metabolically more vulnerable than leukocytes or platelets and may be destroyed prematurely in red pulp.
- Spherocytes may be formed during repeated or prolonged metabolic conditioning in the red pulp.

Symptoms of Splenomegaly

- Splenomegaly may be asymptomatic.
- Very rapid enlargement of the spleen may cause some pain due to strain on the splenic capsule.
- Greatly enlarged spleens may cause abdominal discomfort, trouble sleeping on the left side, and early satiety.
- Splenic infarction may cause pleuritic-like left upper quadrant or shoulder pain, with or without a friction rub.
- In young patients with sickle cell anemia, the spleen may become acutely enlarged and painful due to obstruction of the splenic outflow, with sudden aggravation of anemia (sequestration crisis).

Estimation of Splenic Size

- A spleen of normal size may be palpable in young and thin patients with low diaphragms. Otherwise, a palpable spleen should be considered to be enlarged.
- Splenic size can be assessed with abdominal ultrasound (**Figure 26–1**), computed tomography (CT) (**Figure 26–2**), or magnetic resonance imaging (MRI) examination.
- Cysts, tumors, or infarcts of the spleen may be identified by radionuclide colloid scanning, abdominal CT, or MRI.

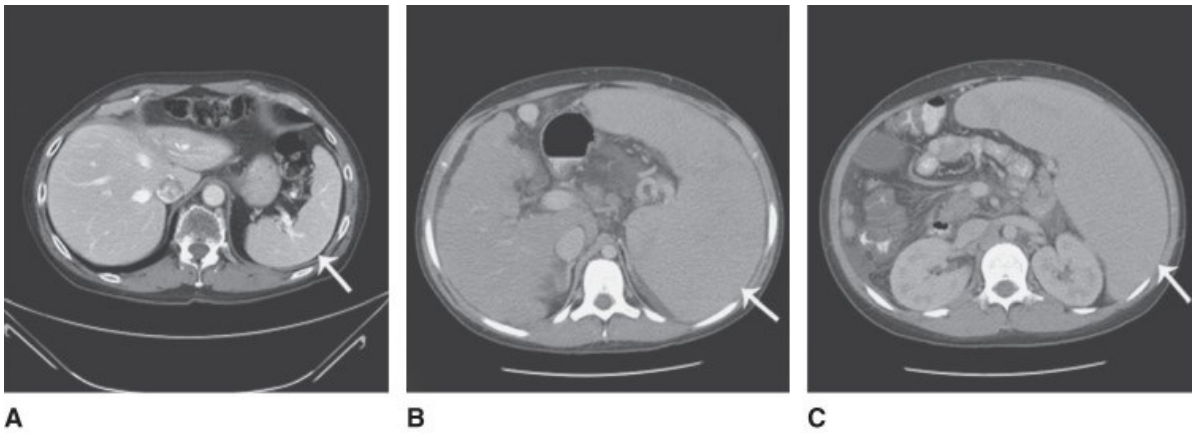


FIGURE 26–1 A three-way composite of abdominal computed tomography. **A.** Normal spleen size. **B.** Enlarged spleen. **C.** Massively enlarged spleen at the level of mid-kidney. Normally the spleen would either not be visualized or only a small lower pole would be evident at the latter level. (*White arrows* mark the edge of the splenic silhouette.) (Used with permission from Deborah Rubens, MD, The University of Rochester Medical Center.)

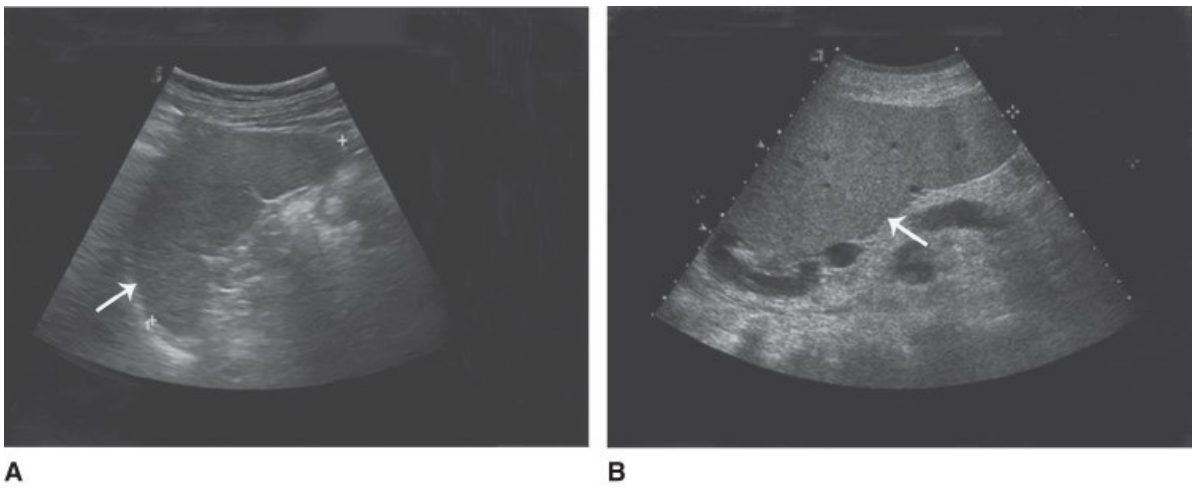


FIGURE 26–2 A two-way composite of ultrasonographic examination for spleen size. Patient's head is to the left side of the longitudinal image. **A.** Image of echo indicating normal spleen size with cranial to caudal longitudinal dimension of 10.3 cm. **B.** Image of echo indicating enlarged spleen with cranial to caudal longitudinal dimension of 16.2 cm. (*White arrows* mark the edge of the splenic silhouette.) The normal spleen is usually less than 13 cm in length, but the examiner has to consider other dimensions in assessing spleen size (volume). (Used with permission from Deborah Rubens, MD, The University of Rochester Medical Center.)

Hematologic Features of Splenomegaly

- The blood concentration of erythrocytes, leukocytes, or platelets is reduced in the blood, with corresponding hyperplasia in the marrow.
- Cellular morphology is usually normal.

Splenectomy

- This procedure may be required for severe, dangerous cytopenias and can lead to dramatic improvement of blood counts, sometimes to normal, in patients with hypersplenism.
- It may alleviate portal hypertension but is not the preferred primary treatment.
- It will alleviate painful splenic infarcts.
- After splenectomy, there may be a rapid, but temporary, increase in the platelet count, which can lead to thromboembolic complications, especially in the elderly or in bedridden patients.
- Chronic changes in the blood after splenectomy are listed below in "Hyposplenism, Laboratory Findings."

- Splenectomy removes a protective filter bed and renders the patient vulnerable to bacteremia, especially due to encapsulated gram-positive organisms. Therefore, vaccination against such organisms (eg, *Streptococcus pneumoniae, Haemophilus influenzae*) should precede elective splenectomy by 2 to 3 weeks if at all possible.
- The procedure diminishes resistance to preexisting parasitic disease (malaria, bartonellosis, babesiosis) and transforms dormant infestation into active disease.
- Partial splenectomy has been used in special circumstances to decrease hypersplenism and prevent hyposplenism.
- The frequency of splenectomy for some disorders has decreased in recent years because of improved alternative therapies or a higher threshold for recommending the procedure.
- Splenectomy is still recommended under specific conditions for some disorders, as discussed in specific chapters (eg, Chap. 13, Erythrocyte Membrane Disorders; Chap. 22, Hemolytic Anemia Resulting from Warm-Reacting Antibodies; Chap. 47, Primary Myelofibrosis, and Chap. 73, Thrombocytopenia). However, as a result of higher risks of overwhelming infection, splenectomy should be postponed, if at all possible, until after age 5.

HYPOSPLENISM (DECREASED SPLENIC FUNCTION)

- Splenic function may be reduced by disease or surgical removal.
- Hyposplenism may or may not be associated with reduced splenic size.
- Impaired filtering causes mild thrombocytosis and increased risk of severe bloodstream infections.
- ^{99m}Tc sulfur colloid uptake is a reliable measure of the capacity of the spleen to clear particulates from the blood.
- Causes of hyposplenism are listed in **Table 26–3**.

TABLE 26–3 CONDITIONS ASSOCIATED WITH HYPOSPLENISM

Miscellaneous
Surgical splenectomy
Splenic irradiation
Sickle hemoglobinopathies
Congenital asplenia
Thrombosis of splenic artery or vein
Normal infants
Gastrointestinal and hepatic diseases
Celiac disease
Dermatitis herpetiformis
Inflammatory bowel disease
Cirrhosis
Autoimmune disorders
Systemic lupus erythematosus
Rheumatoid arthritis
Vasculitis
Glomerulonephritis
Hashimoto thyroiditis
Sarcoidosis
Hematologic and neoplastic disorders
Graft-versus-host disease

Chronic lymphocytic leukemia
Non-Hodgkin lymphoma
Hodgkin lymphoma
Amyloidosis
Advanced breast cancer
Hemangiosarcoma
Sepsis/infectious diseases
Malaria
Disseminated meningococcemia

Source: *Williams Hematology,* 9th ed, Chap. 56, Table 56–3.

Infectious Complications

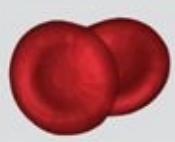
- Overwhelming sepsis is often fatal.
- The condition is usually caused by encapsulated bacteria, such as pneumococcus or *H. influenzae*.
- Risk greatest in very young and splenectomy usually contraindicated before age 4 years.
- Healthy adults with splenectomy because of accidental rupture of normal spleen are still at some increased risk.

Laboratory Findings

- Slight to moderate increase in leukocyte and platelet counts
- Target cells, acanthocytes, and other misshapen erythrocytes
- Howell-Jolly bodies (nuclear fragment remnants) in one red cell per 100 to 1000
- Pitted erythrocytes (wet preparation, using direct interference-contrast microscopy)
- Increased numbers of Heinz bodies on supravital examination
- Increased numbers of nucleated red cells in patients splenectomized for various hemolytic disorders

Treatment of Hyposplenic or Postsplenectomy Patient

- Immunize with polyvalent pneumococcal vaccine before splenectomy.
- Vaccinate children against *H. influenzae*.
- Prophylactic penicillin is usually given to asplenic children.
- All febrile infections should be considered serious. Administer an appropriate antibiotic regimen immediately on onset of symptoms.
- Treat with broad-spectrum antibiotics at the time of all dental work (especially extractions).



For a more detailed discussion, see Jaime Caro and Srikanth Nagalla: Hypersplenism and Hyposplenism, Chap. 56 in *Williams Hematology*, 9th ed.

CHAPTER 27

Polyclonal Polycythemias (Primary and Secondary)

- Polycythemia, also referred to as erythrocytosis, is characterized by an increased red cell mass. There is no consensus on terminology (ie, primary familial polycythemia but postrenal transplantation erythrocytosis). Polycythemias can be primary or secondary and can be inherited or acquired.
- Classification of polycythemic disorders appears in **Table 2–2** in Chap. 2.
- Primary polycythemias are caused by somatic or germline mutations within hematopoietic stem cells or erythroid progenitors that result in an augmented response to erythropoietin. This response is inappropriate; that is, it is not a compensation for hypoxia.
- Secondary polycythemias are caused by either an appropriate (compensatory) or inappropriate increase in the red cell mass as a result of augmented levels of erythropoietin.

PRIMARY POLYCYTHEMIA

- The most common primary polycythemia, polycythemia vera, is a clonal acquired multipotential hematopoietic progenitor cell disorder discussed in Chap. 41. It is a myeloproliferative neoplasms.

Primary Familial and Congenital Polycythemia

- This disorder is autosomal dominant, with normal leukocyte and platelet counts.
- Affected persons may be misdiagnosed as having polycythemia vera.
- Low plasma erythropoietin level is a constant feature (see **Figure 27–1**).
- Erythroid progenitors in in vitro cultures are hypersensitive to erythropoietin, but unlike the erythroid progenitors in polycythemia vera, they do not grow in the absence of erythropoietin.
- This condition is caused by a truncation of erythropoietin receptor and deletion of the negative regulatory cytoplasmic domain.
- Affected individuals may have an increased risk of cardiovascular complications, regardless of control of elevated hematocrit by phlebotomies.

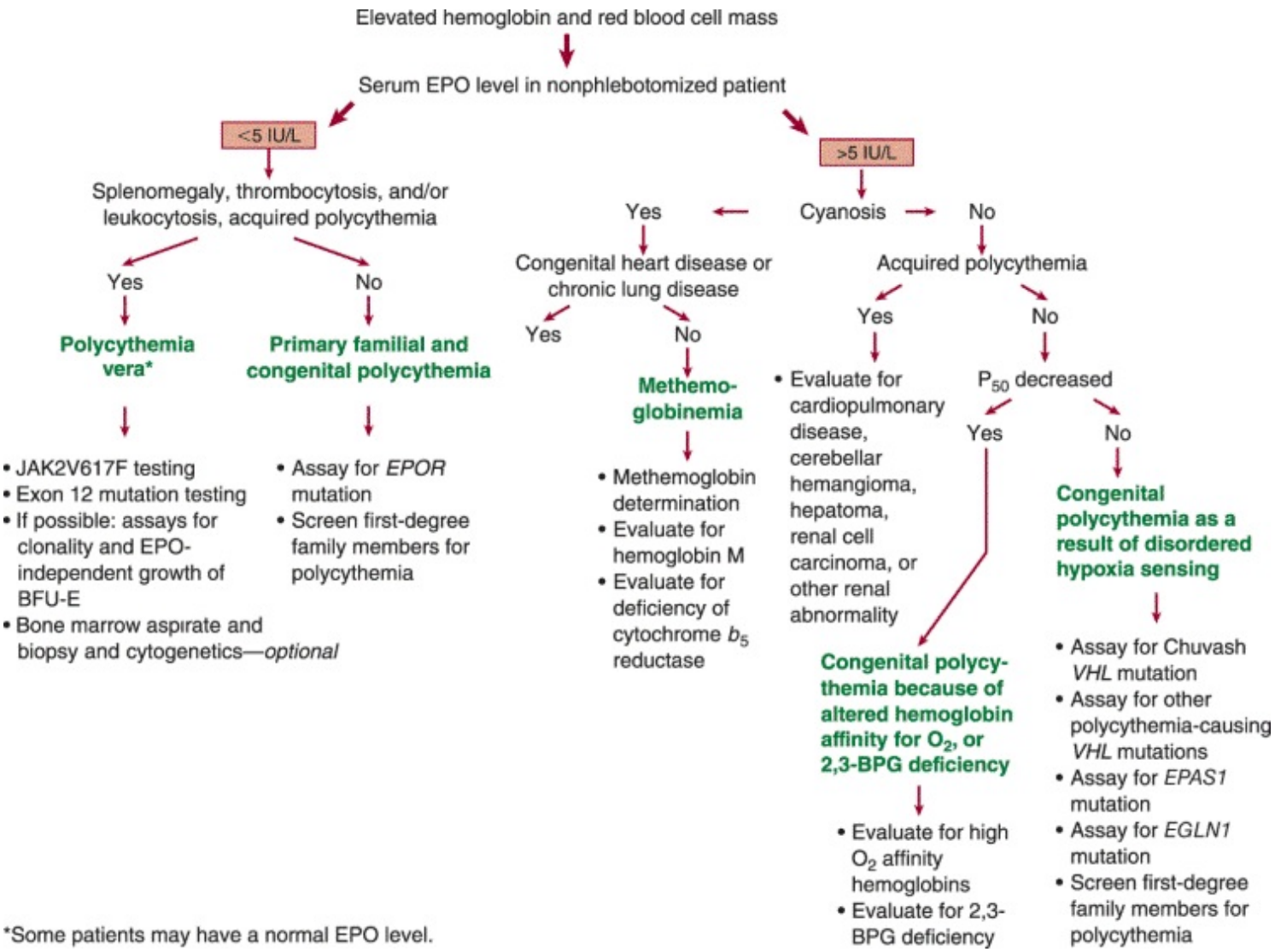


FIGURE 27–1 Diagnostic algorithm for polycythemia based on erythropoietin (EPO) level. BFU–E, burst-forming unit–erythroid; BPG, bisphosphoglycerate. (Source: *Williams Hematology,* 9th ed, Fig. 57–6.)

SECONDARY POLYCYTHEMIA (ERYTHROCYTOSIS)

- This group of disorders is marked by increased red cell mass (absolute polycythemia) because of stimulation of red cell production by increased erythropoietin production. The polycythemia is considered:
  — *Appropriate* if there is tissue hypoxia and the increased red cell mass (increased blood hemoglobin concentration) minimizes the hypoxia.
  — *Inappropriate* if tissue hypoxia is absent and the polycythemia serves no useful purpose.

Appropriate Secondary Polycythemias

High-Altitude Acclimatization

- There is a great variability in an individuals' susceptibility to acute and chronic mountain sickness complications.
- Some populations such as Tibetans and Ethiopian dwellers of high mountains have a genetically determined resistance to these complications, whereas extreme polycythemia is

often seen in Quechuas and Aymaras living at high-altitude (Andean natives).

- Acute mountain sickness involves:
  — Cerebral hypoxia is causal, and the condition may be life-threatening. Polycythemia does not occur.
  — Affected persons may have headaches, insomnia, palpitations, weakness, nausea, vomiting, and mental dullness, and they may develop pulmonary and cerebral edema.
  — Treatment is with oxygen, dexamethasone, and acetazolamide and if feasible rapid return to lower altitude.
- Chronic mountain sickness involves:
  — This condition occurs after prolonged exposure to high altitudes; there can be a genetic predisposition.
  — It is characterized by marked polycythemia, cyanosis, plethora, pulmonary hypertension, clubbing of the fingers, and signs of right heart failure.
  — Treatment with the angiotensin-converting enzyme inhibitor enalapril may be effective.
  — Iron deficiency (often induced by misguided phlebotomies) leads to deterioration of pulmonary hypertension.
  — A return to a normal state develops slowly after descent to lower altitude.

Pulmonary Disease

- This is associated with arterial oxygen desaturation cyanosis and clubbing.
- In chronic obstructive pulmonary disease, chronic inflammation and infection may blunt erythropoietin synthesis and secretion and compensatory red cell production.
- Venesection is controversial; many consider it ill-advised, but some recommend it to maintain the hematocrit at no more than 55%, presumably to optimize oxygen-carrying capacity as a result of lower blood viscosity and more optimal blood flow characteristics. The use of phlebotomy has not been shown to be useful in a clinical trial.

Alveolar Hypoventilation

- Central form may be a result of cerebral vascular accident, parkinsonism, encephalitis, or barbiturate intoxication.
- Peripheral form may be a result of myotonic dystrophy, poliomyelitis, spondylitis, or severe obesity.

Sleep Apnea

- Only a minority (< 5%) of subjects develop erythrocytosis. This finding is as yet unexplained.

Cardiovascular (Eisenmenger Syndrome)

- In patients with congenital right-to-left intracardiac shunts, arterial PO_2 decreases significantly, erythropoietin secretion increases, and the hematocrit may reach 75% to 85%.
- Reduction of the hematocrit by phlebotomy to improve blood flow may not be beneficial, and such therapy is controversial.
- Treatment with phlebotomy is indicated for cerebral symptoms (headaches, difficulty to concentrate); however, if prompt improvement after phlebotomies does not ensue, phlebotomies are probably of no benefit.

- Dehydration should be avoided to prevent further increase in hematocrit.
- Other right-to-left shunts can result in secondary polycythemia in hepatic cirrhosis (pulmonary arteriovenous or portopulmonary venous shunts), hereditary hemorrhagic telangiectasia, and idiopathic pulmonary arteriovenous aneurysms.

Inappropriate Secondary Polycythemias

Acquired High-Affinity Hemoglobinopathy

- This may be a result of elevated blood carboxyhemoglobin (smoking).

Toxic Dysregulation of Hypoxia Sensing

- Cobalt chloride treatment inhibits principal negative regulator of hypoxia-inducible factors (HIFs) (ie, prolyl hydroxylase 2) and leads to an increased hematocrit. Particularly high hematocrits (as high as 90%) are recorded in cobalt miners in Peruvian mines in the high Andes.

Postrenal Transplantation Erythrocytosis

- Such erythrocytosis is defined as a persistent elevation of the hematocrit of more than 51%.
- It is found in approximately 5% to 10% of renal allograft recipients; incidence may be decreasing because of widespread use of angiotensin-converting enzyme inhibitors.
- It develops within 8 to 24 months after transplantation, despite good function of the allograft.
- Therapy with either angiotensin-converting enzyme inhibitor enalapril or with the angiotensin II receptor type 1 blocker losartan is generally effective.

Renal Cysts and Hydronephrosis

- Erythropoietin can be demonstrated in cyst fluid or is due to cyst-induced mechanical renal ischemia downstream of the cyst.

Renal Tumors

- One percent to 3% of patients with hypernephroma have erythrocytosis, probably as a consequence of excess erythropoietin formed by the tumor.
- Remission of erythrocytosis occurs after tumor removal.
- Reappearance of erythrocytosis heralds recurrence.
- These may be associated with von Hippel-Lindau (*VHL*) gene mutation.

Cerebellar Hemangiomas

- About 15% of patients have erythrocytosis, and erythropoietin can be demonstrated in cyst fluid and stromal cells.
- These may be associated with *VHL* gene mutations.

Other Tumors

- These are uterine myomas, usually huge. Treatment is removal of the myoma routinely followed by return to normal hemoglobin concentration.
- Hepatoma can cause erythrocytosis, probably because of erythropoietin production by the

neoplastic cells.

Endocrine Disorders

- Pheochromocytoma, aldosterone-producing adenomas, Bartter syndrome, or dermoid cyst of ovary may be associated with increased erythropoietin levels and erythrocytosis, which respond to removal of the tumor.
- Pheochromocytoma may be associated with *VHL* or other gene mutations (see Chap. 6).
- In Cushing syndrome, cortisol and other corticosteroids may cause general marrow stimulation and mild erythrocytosis.

Androgen Usage

- Androgens of the 5α-H configuration stimulate erythropoietin production and result in erythrocytosis.
- Androgens of the 5α-H configuration also enhance differentiation of stem cells.

Neonatal Erythrocytosis

- This is a normal physiologic response to intrauterine hypoxia and high oxygen-affinity fetal hemoglobin.
- It may be excessive in infants of diabetic mothers.
- Late cord clamping may be contributory.
- Partial exchange transfusion is sometimes performed if the hematocrit is above 65% at birth.

Autotransfusion (Blood Doping)

- Autotransfusion of stored red cells prior to competition improves performance in cross-country skiers and long-distance runners but at the risk of life-threatening hyperviscosity when associated with fluid losses from strenuous activity.
- This should be suspected when an elevated hematocrit is associated with very low level of erythropoietin in an athlete.
- Injection of commercial erythropoietin preparations will achieve the same effect as autotransfusion. This approach, in addition to being unethical to improve athletic performance, bears the risk of overdose and life-threatening hyperviscosity under periods of athletic stress and dehydration, as well as from other nonerythroid cardiovascular effects of high erythropoietin levels.

Congenital Secondary Polycythemias

Hereditary High-Affinity Hemoglobins

- Inheritance is autosomal dominant.
- Only about 50% of the abnormal hemoglobins are demonstrable by hemoglobin electrophoresis. Thus, the initial and the only appropriate test is determination of hemoglobin-oxygen affinity (estimated by measuring the p50) determined by co-oximeter. If a co-oximeter is not available for measurement of full hemoglobin-oxygen dissociation kinetics, p50 can be calculated from venous blood (see Chap. 18).
- Increased hemoglobin-oxygen affinity (decreased p50) results in tissue hypoxia; erythropoietin

may be high or normal.
- Phlebotomies are generally ill advised unless severe symptoms of hyperviscosity.

2,3-Biphosphoglycerate Deficiency

- This deficiency results in an increased oxygen affinity of hemoglobin (decreased p50).
- It is caused by bisphosphoglyceromutase deficiency (see Chaps. 14 and 18).

Congenital Methemoglobinemias

- Mild polycythemia occurs in patients with methemoglobinemia caused by recessively inherited cytochrome b_5 reductase deficiency (see Chap. 14) or globin mutations causing dominantly inherited methemoglobinemia (see Chap. 18).

Congenital Disorders of Hypoxia Sensing

Chuvash Polycythemia

- This disorder is endemic in the Chuvash autonomous region of Russia and the Italian island of Ischia; it is sporadic worldwide.
- Inheritance is autosomal recessive.
- The cause is mutation in the von Hippel-Lindau gene (*VHL* C598T) that upregulates HIF transcription factors that increase transcription of many genes, including erythropoietin.
- Erythropoietin levels are normal or increased.
- Erythroid progenitors in in vitro cultures are hypersensitive to erythropoietin, thus sharing features of both primary and secondary polycythemia.
- Strokes and other thrombotic vascular complications and pulmonary hypertension lead to early mortality and are not ameliorated by phlebotomies.

Congenital Polycythemia from Other *VHL* Gene Mutations

- Most patients are compound heterozygotes for Chuvash *VHL* C598T and other VHL gene mutation.
- Rare patients have only a single *VHL* mutation.

Prolyl Hydroxylase Deficiency

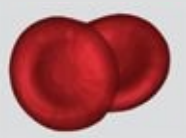
- Rare recessive loss-of-function mutation of *EGLN1* gene (encoding prolyl hydroxylase 2) disorder causing mild or borderline polycythemia associated with upregulated HIFs.
- Because of its rarity, little is known about its clinical manifestations.

HIF-2α Gain-of-Function Mutations

- This rare disorder is due to gain-of-function mutations of *EPAS1* gene (encoding HIF-2α), leading to increased activity of HIF-2 that increases transcription of erythropoietin.
- Its genetic mosaicism may be also associated with congenital polycythemia and at onset of pheochromocytoma and other tumors (see Chap. 6).
- Other clinical manifestations are being defined.

Apparent Polycythemia (Relative or Spurious Polycythemia)

- This condition is characterized by an increased hematocrit, normal red cell mass, and low plasma volume.
- In the past, it was referred to as Gaisbock syndrome; pseudo-polycythemia; or stress, spurious, and smokers' polycythemia.
- It is associated with obesity, hypertension, use of diuretics, and smoking.
- Differential diagnosis includes severe dehydration.
- Treatment should be directed toward any underlying condition, if present, such as obesity (weight reduction) or cigarette smoking (cessation of smoking).



For a more detailed discussion, see Josef T. Prchal and Perumal Thiagaragan: Erythropoiesis, Chap. 32; Archana M. Agarwal and Josef T. Prchal: Methemoglobinemia and other dyshemoglobinemias, Chap. 50; Josef T. Prchal: Primary and Secondary Polycythemias (Erythrocytosis), Chap. 57 in *Williams Hematology*, 9th ed.

CHAPTER 28
The Porphyrias

- The porphyrias are inherited or acquired disorders in which the activity of an enzyme in the heme biosynthetic pathway is altered. Metabolic intermediates are produced in excess, initially either in the marrow or the liver, and result in neurologic and/or photocutaneous symptoms and signs.

CLASSIFICATION

- See **Table 28–1**.
- The two organs most active in heme biosynthesis are the marrow and the liver. Photosensitivity (indicated below with the following symbols as either **blistering*** or **nonblistering**†) and/or (indicated below with the following symbol as) **neurovisceral symptoms**‡ may be part of the porphyria phenotype. Therefore, porphyrias are classified as erythropoietic or hepatic and as cutaneous or acute.

TABLE 28–1 HUMAN PORPHYRIAS: SPECIFIC ENZYMES AFFECTED BY MUTATIONS, MODES OF INHERITANCE, CLASSIFICATION, AND MAJOR CLINICAL FEATURES OF EACH OF THE HUMAN PORPHYRIAS

Porphyria[a]	Affected Enzyme	Known Mutations	Inheritance	Classification	Principal Clinical Features
X-linked protoporhyria (XLP)	δ-Aminolevulinic acid (ALA) synthase erythroid-specific form (ALAS2)	4 (gain of function)	Sex-linked recessive	Erythropoietic	Nonblistering photosensitivity
δ-Aminolevulinic acid dehydratase porphyria (ADP)	ALA dehydratase (ALAD)	10	Autosomal recessive	Hepatic[b]	Neurovisceral
Acute intermittent porphyria (AIP)	PBG deaminase (PBGD)	273	Autosomal dominant	Hepatic	Neurovisceral
Congenital erythropoietic porphyria (CEP)	Uroporphyrinogen III synthase (UROS)	36	Autosomal recessive	Erythropoietic	Neurovisceral
Porphyria cutanea tarda (PCT)	Uroporphyrinogen decarboxylase (UROD)	70 (includes HEP)	Autosomal dominant[c]	Hepatic	Blistering photosensitivity
Hepatoerythropoietic porphyria (HEP)	UROD	—	Autosomal recessive	Hepatic[b]	Blistering photosensitivity
Hereditary coproporphyria (HCP)	Coproporphyrinogen oxidase (CPO)	42	Autosomal dominant	Hepatic	Neurovisceral; blistering photosensitivity

					(uncommon)
Variegate porphyria (VP)	Protoporphyrinogen oxidase (PPO)	130	Autosomal dominant	Hepatic	Neurovisceral; blistering photosensitivity (common)
EPP – classic form	Ferrochelatase (FECH)	90	Autosomal recessive[d]	Erythropoietic	Nonblistering photosensitivity

[a]Porphyrias are listed in the order of the affected enzyme in the heme biosynthetic pathway.

[b]These porphyrias also have erythropoietic features, including increases in erythrocyte zinc protoporphyrin.

[c]Heterozygous UROD mutations are present in familial (type 2) but not in the more common sporadic (type 1) PCT. In all cases, an acquired inhibition of hepatic UROD reduces the enzyme activity to less than ~20% of normal.

[d]Because both alleles are abnormal in affected individuals (in most cases with a severe FECH mutation trans to a hypomorphic FECH allele), EPP is now regarded as recessive at the molecular level.

Source: *Williams Hematology,* 9th ed, Chap. 58, Table 58–1.

Erythropoietic Porphyrias

- Principal site of initial accumulation of pathway intermediates: the erythroblast
- Congenital erythropoietic porphyria (CEP)*
- Erythropoietic protoporphyria (EPP)†
- X-linked protoporphyria (XLP)†

Hepatic Porphyrias

- Principal site of initial accumulation of pathway intermediates: the liver
- δ-Aminolevulinic acid dehydratase porphyria (ADP)‡
- Acute intermittent porphyria (AIP)‡
- Hereditary coproporphyria (HCP)*‡
- Variegate porphyria (VP)*‡
- Porphyria cutanea tarda (PCT)*
- Hepatoerythropoietic porphyria (HEP)*

SPECIFIC DISORDERS

General Considerations

- Synthesis of heme is catalyzed by a series of eight enzymes. Altered activity of each of these enzymes is associated with a specific form of porphyria (see **Figure 28–1**).
- Diagnostic biochemical findings in the individual porphyrias are summarized in **Table 28–2**.

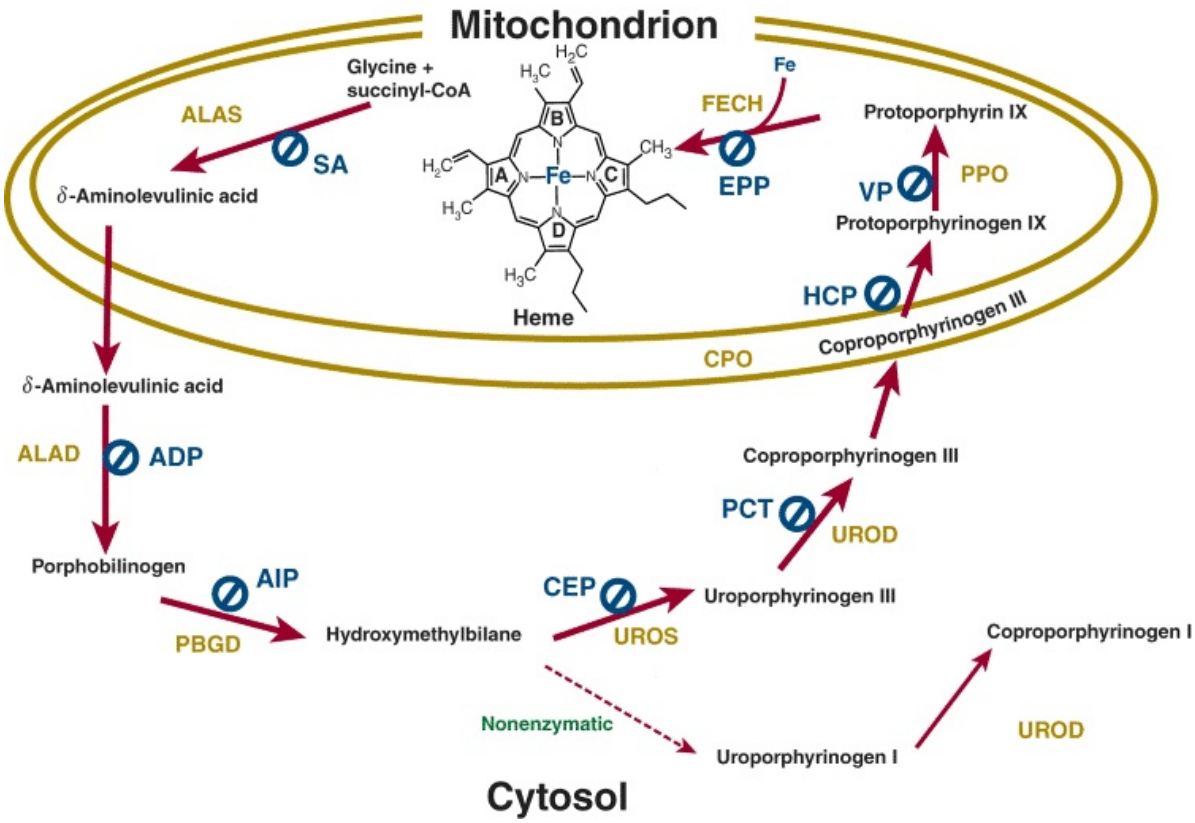


FIGURE 28–1 Enzymes and intermediates in the heme biosynthetic pathway and the type of porphyria associated with a deficiency of each enzyme (indicated by Ø). Gain of function mutation of the erythroid form of ALA synthase is not shown. Abbreviations: ADP, ALA dehydratase porphyria; AIP, acute intermittent porphyria; ALAD, δ-aminolevulinic acid dehydratase; ALAS, δ-aminolevulinic acid synthase; CEP, congenital erythropoietic porphyria; CPO, coproporphyrinogen oxidase; EPP, erythropoietic protoporphyria; FECH, ferrochelatase; HCP, hereditary coproporphyria; PBGD, porphobilinogen deaminase; PCT, porphyria cutanea tarda; PPO, protoporphyrinogen oxidase; SA, sideroblastic anemia; UROD, uroporphyrinogen decarboxylase; UROS, uroporphyrinogen III synthase; VP, variegate porphyria. (Source: *Williams Hematology*, 9th ed, Chap. 58, Fig. 58–1.)

TABLE 28–2 BIOCHEMICAL FINDINGS INCLUDING MAJOR INCREASES IN PORPHYRINS AND PORPHYRIN PRECURSORS IN THE HUMAN PORPHYRIAS[a]

Porphyria	Erythrocytes	Plasma	Urine	Stool
XLP	Metal-free and zinc protoporphyrin[e]	Protoporphyrin (-634 nm)[d]	[f]	Protoporphyrin[b]
ADP	Zinc protoporphyrin	ALA[b]	ALA, coproporphyrin III	[b]
AIP	Decreased PBGD activity (most cases)[b]	ALA, PBG[b] (~620 nm), some cases[c]	ALA, PBG, uroporphyrin	[b]
CEP	Uroporphyrin I; coproporphyrin I	Uroporphyrin I, coproporphyrin I (~620 nm)[c]	Uroporphyrin I; coproporphyrin I	Coproporphyrin I
PCT and HEP	Zinc protoporphyrin (in HEP)	Uroporphyrin, heptacarboxyl porphyrin (~620 nm)[c]	Uroporphyrin, heptacarboxyl porphyrin	Heptacarboxyl porphyrin, isocoproporphyrins
HCP	[b]	[d] (~620 nm, some cases)[c]	ALA, PBG, coproporphyrin III	Coproporphyrin III
VP	[b]	Protoporphyrin (~628 nm)[c]	ALA, PBG, coproporphyrin III	Coproporphyrin III, protoporphyrin

EPP	Metal free protoporphyrin[e]	Protoporphyrin[e] (~634 nm)[c]	[f]	Protoporphyrin[b]

Abbreviations: ADP, ALA dehydratase porphyria; AIP, acute intermittent porphyria; ALA, δ-aminolevulinic acid; HEP, Hepatoerythropoietic porphyria; CEP, congenital erythropoietic porphyria; EPP, erythropoietic protoporphyria; HCP, hereditary coproporphyria; PBG, porphobilinogen; PBGD, porphobilinogen deaminase; PCT, porphyria cutanea tarda; VP, variegate porphyria.

[a]Porphyrias are listed in the order of the affected enzyme in the heme biosynthetic pathway.

[b]Porphyrin levels normal or slightly increased.

[c]Fluorescence emission peak of diluted plasma at neutral pH.

[d]Plasma porphyrins usually normal, but increased when blistering skin lesions develop.

[e]Zinc protoporphyrin ≤ 15% of total in XLP, but 15%–50% in variant form.

[f]Urine porphyrins (especially coproporphyrin) increase only with hepatopathy.

Source: *Williams Hematology,* 9th ed, Chap. 58, Table 58–2.

ERYTHROPOIETIC PORPHYRIAS

Congenital Erythropoietic Porphyria

Pathogenesis

- This rare (~200 cases reported) autosomal recessive disorder is caused by an almost complete (< 5% of normal) deficiency of uroporphyrinogen III synthase activity.

Clinical Findings

- Cutaneous photosensitivity appears early in life. Subepidermal bullous lesions develop and progress to crusted erosions that heal with scarring, pigmentary changes, hypertrichosis, and alopecia. Bacterial infections contribute to mutilation of facial features and fingers.
- This condition may present before birth as fetal hydrops.
- Red teeth with red fluorescence under ultraviolet (UV) light is characteristic.
- Hemolytic anemia is common, with splenomegaly and compensatory marrow expansion.
- Late-onset cases may be associated with clonal myeloproliferative or myelodysplastic disorders.

Diagnosis and Laboratory Findings

- In utero, dark brown porphyrin-rich amniotic fluid is characteristic.
- In newborns, pink or dark brown staining of diapers may suggest the diagnosis.
- Erythrocyte and urinary porphyrins (predominantly uroporphyrin and coproporphyrin, isomer I), and fecal porphyrins (predominantly coproporphyrin I) are markedly increased.
- Mutations should be confirmed by DNA studies in all cases.

Treatment

- Avoid sunlight and skin trauma and treat infections promptly. Topical sunscreens that block UVA and visible light are of little value.
- Hematopoietic stem cell transplantation in early childhood is most effective.
- Suppression of marrow with hypertransfusion or hydroxyurea, splenectomy, and oral charcoal have been of limited value.

Erythropoietic Protoporphyria and X-linked Protoporphyria

Pathogenesis

- Erythropoietic protoporphyria (EPP), the third most common porphyria and the most common in children, is caused either by loss of function mutations of ferrochelatase (FECH) or gain of function mutations of the erythroid form of δ-aminolevulinic acid synthase (ALAS) ([ALAS2]). At the molecular level, EPP is an autosomal recessive disorder (with both FECH alleles affected by loss-of-function mutations) or X-linked (gain of function mutation of one ALAS2 allele).
- In EPP, functional deficiency in the enzyme to less than ~20% of normal results from a severe FECH mutation trans to a common hypomorphic FECH allele. Occasionally, severe mutations affect both FECH alleles.
- In the X-linked protoporphyria, a truncated ALAS2 allele leads to gain of function, resulting in significantly increased production of δ-ALA, which is metabolized to protoporphyrin IX in erythroblasts.

Clinical Findings

- Childhood onset of nonblistering cutaneous photosensitivity is characteristic.
- Symptoms and signs include burning pain, itching, redness, and swelling of the skin soon after light exposure (**Table 28–3**).
- Gallstones containing protoporphyrin and presenting at an early age are common.
- There are no neurovisceral symptoms.
- Possible features may include impaired iron absorption and mild microcytic anemia.
- Cholestatic liver disease (protoporphyric hepatopathy), which may present with abdominal pain and jaundice and may progress rapidly but develops in less than 5% of cases.
- Late-onset cases of EPP may be associated with myeloproliferative or myelodysplastic disorders.

TABLE 28–3 COMMON CLINICAL FEATURES OF ERYTHROPOIETIC PROTOPORPHYRIA

Symptoms and Signs	Incidence (% of Total)
Burning	97
Edema	94
Itching	88
Erythema	69
Scarring	19
Vesicles	3
Anemia	27
Cholelithiasis	12
Abnormal liver function results	4

Data from Bloomer J, Wang Y, Singhal A, et al: Molecular studies of liver disease in erythropoietic protoporphyria. *J Clin Gastroenterol* 2005 Apr;39 (4 suppl 2:S167-S175.

Diagnosis and Laboratory Findings

- Diagnosis is more delayed than in any other type of porphyria in part because symptoms out of

proportion to physical findings, and urine porphyrins are normal.
- Excess concentrations of protoporphyrin occur in red cells, plasma, bile, and feces.
- Diagnosis is established by finding marked increase in total erythrocyte protoporphyrin with a predominance for metal-free protoporphyrin rather than zinc protoporphyrin.

Treatment
- Therapy includes avoidance of sun exposure, use of topical sunscreens that block UVA and visible light, oral β-carotene (120–180 mg/d), and afamelanotide (approved in Europe).
- Protoporphyric hepatopathy is treated with erythrocyte transfusions, plasmapheresis, hemin, cholestyramine, ursodeoxycholic acid and vitamin E, and liver and marrow transplantation may be necessary.

HEPATIC PORPHYRIAS

Aminolevulinic Acid Dehydratase Porphyria

Pathogenesis
- This autosomal recessive disorder is due to a severe deficiency of ALA dehydratase.

Clinical Findings
- This is the rarest form of porphyria (six documented cases).
- Patients have neurovisceral symptoms similar to those of AIP (see below).

Laboratory Findings
- Urine ALA and coproporphyrin III excretion is markedly increased; porphobilinogen (PBG) excretion is normal or only slightly increased. Erythrocyte zinc protoporphyrin is markedly increased.
- Red cell ALA dehydratase activity of less than 5% of normal.
- It is necessary to distinguish from other causes of ALA dehydratase deficiency, such as lead poisoning (measure blood lead) and hereditary tyrosinemia I (measure succinylacetone in urine), and identify the causative ALA dehydratase mutations.

Treatment
- The same approach as for AIP is used; hemin appears to be most effective.

Acute Intermittent Porphyria

Pathogenesis
- This autosomal dominant disorder is caused by partial deficiency of porphobilinogen deaminase.

Clinical Findings
- Symptoms usually occur as neuropathic acute attacks lasting for days or if not treated, for weeks.

- Abdominal pain is the most common and often the initial symptom.
- Extremity pain, nausea, vomiting, constipation or diarrhea, abdominal distention, ileus, urinary retention are frequently present.
- Abdominal tenderness, fever, and leukocytosis are usually not prominent.
- Neuropathy, predominantly motor may lead to quadraparesis, respiratory impairment, and bulbar paralysis.
- Seizures (sometimes associated with hyponatremia and inappropriate antidiuretic hormone secretion) and mental symptoms indicate central nervous system involvement.
- Tachycardia, hypertension, sweating and tremors indicate sympathetic overactivity.
- Pain and depression may become chronic.
- **Up to 90% of individuals with decreased PBG deaminase activity remain asymptomatic.**
- Attacks may be precipitated by:
  — Drugs and hormones (especially progesterone) that induce hepatic ALAS1 and cytochrome P450 enzymes
  — Reduced caloric or carbohydrate intake
  — Intercurrent illnesses, infection, or surgery
- Increased risk of hepatocellular carcinoma
- Some drugs (**Table 28–4**)

TABLE 28–4	SOME DRUGS CONSIDERED UNSAFE IN ACUTE PORPHYRIAS[a]
Alcohol	
Barbiturates[a]	
Carbamazepine[a]	
Carisoprodol[a]	
Clonazepam (high doses)	
Danazol[a]	
Diclofenac[a] and possibly other NSAIDs	
Ergots	
Estrogens[a,b]	
Ethchlorvynol[a]	
Glutethimide[a]	
Griseofulvin[a]	
Mephenytoin	
Meprobamate[a] (also mebutamate[a], tybamate[a])	
Methyprylon	
Metoclopramide[a]	
Phenytoin[a]	
Primidone[a]	
Progesterone and synthetic progestins[a]	
Pyrazinamide[a]	

Pyrazolones (aminopyrine, antipyrine)
Rifampin[a]
Succinimides (ethosuximide, methsuximide)
Sulfonamide antibiotics[a]
Valproic acid[a]

NSAIDs, nonsteroidal anti-inflammatory drugs.

[a]Porphyria is listed as a contraindication, warning, precaution, or adverse effect in U.S. labeling for these drugs.

[b]Estrogens are unsafe for porphyria cutanea tarda, but can be used with caution in the acute porphyrias.

Note: More complete sources, such as the websites of the American Porphyria Foundation (www.porphyriafoundation.com) and the European Porphyria Initiative (www.porphyria-europe.com), should be consulted before using drugs not listed here.

Adapted with permission from Anderson KE, Bloomer JR, Bonkovsky HL, et al: Recommendations for the diagnosis and treatment of the acute porphyrias, *Ann Intern Med*. 2005 Mar 15;142(6):439-50.

Diagnosis and Laboratory Findings

- Urine may be dark (porphobilin) or red (porphyrins).
- Rapid screening for a substantial increase in urinary PBG is recommended.
- ALA and PBG concentrations in urine are typically ALA 25 to 100 mg/d and PBG 50 to 200 mg/d during attacks (see **Figure 28–2**).
- Decreased PBG deaminase activity (~50% of normal) occurs in ~90% of cases.
- Diagnosis should be confirmed by finding the disease-causing mutation, which is often family-specific.

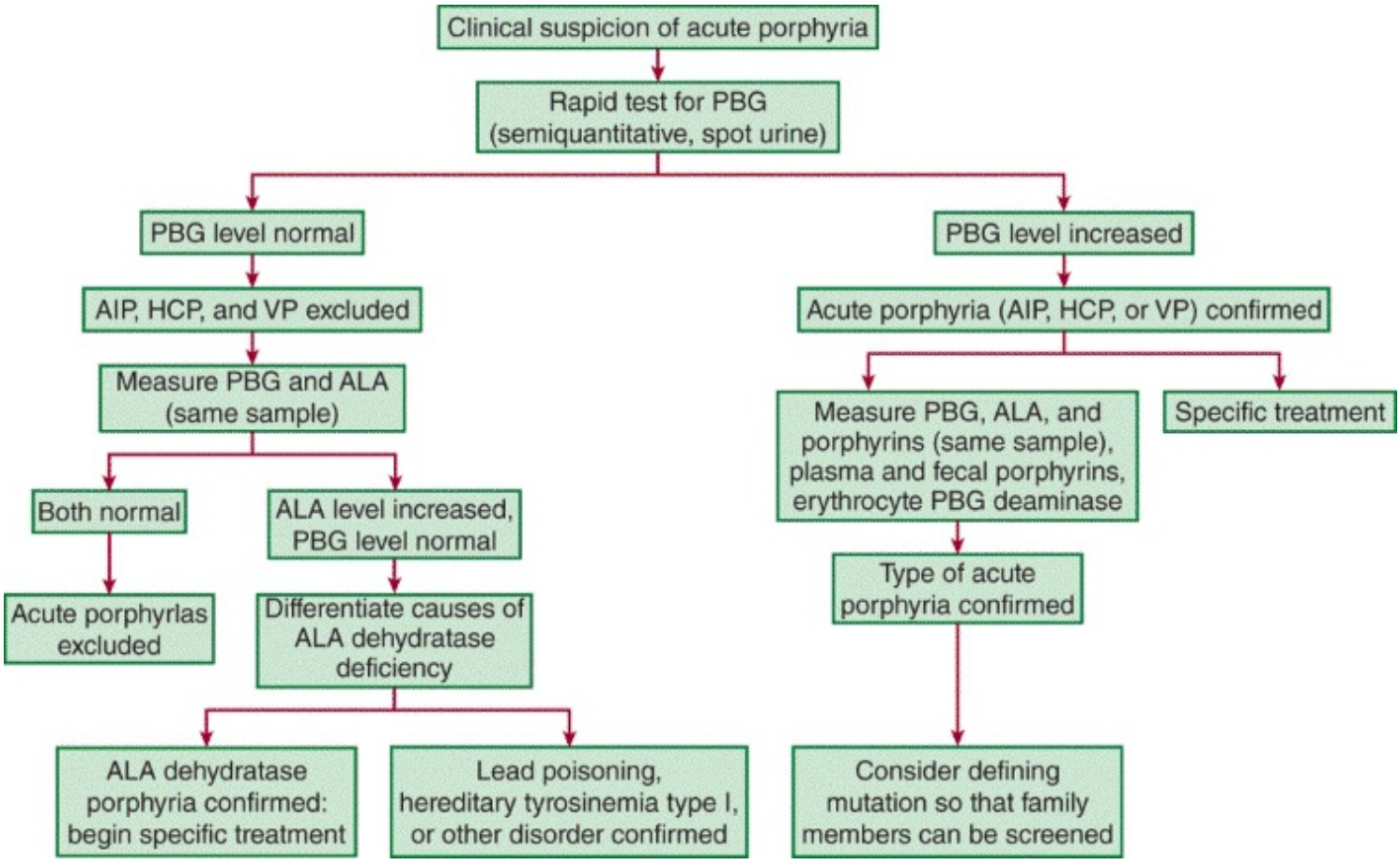


FIGURE 28–2 Recommended laboratory evaluation of patients with concurrent symptoms suggesting an acute porphyria, indicating how the diagnosis is established or excluded by biochemical testing and when specific therapy should be initiated. This

schema is not applicable to patients who have been recently treated with hemin or who have recovered from past symptoms suggestive of porphyria. Levels of δ-aminolevulinic acid (ALA) and porphobilinogen (PBG) may be less increased in hereditary coproporphyria (HCP) and variegate porphyria (VP) and decrease more quickly with recovery than in acute intermittent porphyria (AIP). Mutation detection provides confirmation and greatly facilitates detection of relatives with latent porphyria. (Source: *Williams Hematology*, 9th ed, Chap. 58, Fig. 58–6.)

Treatment

- Ensure adequate caloric intake.
- Avoid precipitating drugs (an up-to-date list is available at http://www.porphyriafoundation.com/testing-and-treatment/drug-safety-in-acute-porphyria).
- Initiate prompt treatment of fasting, intercurrent disease, or infection.
- Acute attacks usually require admission to hospital:
  — Mild attacks may be treated by glucose loading (at least 300 g/d intravenously).
  — Intravenous hemin (3–4 mg/kg once daily for 4 days) is the treatment of choice for all but mild attacks. Reconstitution with human albumin rather than sterile water is recommended to prevent infusion-site phlebitis.
- Long-acting agonists of gonadotrophic-releasing hormone can be effective in preventing frequent premenstrual attacks in women.
- Liver transplantation can be curative in patients who become refractory to other therapies.
- Liver imaging at 6- to 12-month intervals is recommended to screen for early hepatocellular carcinoma in all acute porphyrias.

Hereditary Coproporphyria

- This autosomal dominant disorder is caused by partial deficiency of coproporphyrinogen oxidase.

Clinical Findings

- Neurovisceral manifestations are the same as in AIP.
- Attacks are precipitated by the same factors as in AIP.
- Blistering skin lesions resembling PCT may occur but much less commonly than in VP.
- Increased risk of hepatocellular carcinoma.

Diagnosis and Laboratory Findings

- Excessive urinary ALA, PBG, and uroporphyrin occur during attacks, as in AIP. Urine coproporphyrin III is also increased.
- This disorder is distinguished from AIP by marked increase in fecal porphyrins with a predominance of coproporphyrin III.
- DNA studies can identify the causative coproporphyrinogen oxidase (CPO) mutation in almost all cases.

Treatment

- Therapy is the same as in AIP.

Variegate Porphyria

Pathogenesis

- This autosomal dominant disorder is caused by partial deficiency of protoporphyrinogen oxidase.

Clinical Findings

- Neurovisceral manifestations are the same as in AIP and HCP.
- Attacks are precipitated by the same factors as in AIP and HCP.
- Blistering skin lesions resembling PCT are common and may occur apart from the neurovisceral symptoms.
- There is an increased risk of hepatocellular carcinoma.

Laboratory Findings

- Excessive urinary ALA, PBG, and uroporphyrin occur during attacks, as in AIP. Urine coproporphyrin III is also increased.
- This type of porphyria is distinguished from AIP and HCP by marked increase in fecal porphyrins with a predominance of both coproporphyrin III and protoporphyrin IX.
- Presence of increased plasma porphyrins with a fluorescence emission peak at ~628 nm is diagnostic.
- DNA studies can identify the causative PPO mutation in almost all cases.

Treatment

- Neurovisceral attacks are treated as in AIP.
- Protective clothing and avoidance of sunlight are important in patients with blistering photosensitivity. Treatments for PCT are not effective in VP.

Porphyria Cutanea Tarda

Pathogenesis

- PCT is the most common human porphyria.
- It results from a substantial deficiency of hepatic uroporphyrinogen (URO) decarboxylase (UROD) activity, which is due to generation of an inhibitor (uroporphomethene).
- PCT is an iron-related disorder, with hepatic siderosis in almost all cases.
- The majority of patients have at least several of the following susceptibility factors are found: ethanol use, smoking, estrogen use (in females), hepatitis C, HFE mutations, HIV infection, and UROD mutations.
- Most patients do not have UROD mutations and are referred to as type 1 (or type 3 if additional family members have PCT).
- Type 2 patients are heterozygous for UROD mutations, sometimes have affected relatives or earlier onset of disease, but are otherwise indistinguishable from type 1.

Clinical Findings

- Patients have increased skin fragility and blistering of sun exposed areas, especially the backs of the hands.
- Other findings may include hyperpigmentation, hypertrichosis, alopecia, and scarring.

- Cirrhosis and increased risk of hepatocellular carcinoma may result from one or more susceptibility factors or PCT itself.
- Outbreaks due to environmental or occupational exposure to halogenated cyclic aromatic hydrocarbons, such as hexachlorobenzene, have been reported.

Diagnosis and Laboratory Findings

- Levels of highly carboxylated porphyrins, especially uroporphyrin and heptacarboxyl porphyrin are markedly increased in urine and plasma.

Treatment

- Susceptibility factors should be identified, and exposures to alcohol and estrogens discontinued. Hepatitis C should be treated later.
- Repeated phlebotomy to reduce the serum ferritin to ~20 ng/mL is the preferred treatment and is highly effective.
- A low-dose regimen of hydroxychloroquine (100 mg twice weekly) is also effective when phlebotomies are contraindicated or poorly tolerated.

Hepatoerythropoietic Porphyria

Pathogenesis

- HEP is a rare disorder caused by homozygous or compound heterozygosity for mutations in the URO decarboxylase gene. This is the homozygous form of familial (type 2) PCT, but at least one allele must express some enzyme activity.

Clinical Findings

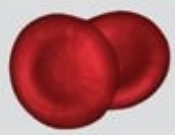
- HEP is characterized by childhood onset and is clinically similar to CEP.
- Anemia and hepatosplenomegaly may be present.

Diagnosis and Laboratory Findings

- Porphyrin elevations in urine, serum, and feces resemble PCT, but in addition there is marked elevation of erythrocyte zinc protoporphyrin.
- Erythrocyte URO decarboxylase activity is reduced to 2% to 10% of normal.

Treatment

- Avoidance of sun exposure is most important; topical sunscreens that block UVA and visible light may be of some benefit.
- Treatments used in PCT are generally not effective.



For a more detailed discussion, see John D. Phillips and Karl Anderson: The Porphyrias, Chap. 58 in *Williams Hematology*, 9th ed.

PART III

DISORDERS OF GRANULOCYTES

CHAPTER 29

Classification and Clinical Manifestations of Neutrophil Disorders

GENERAL CONSIDERATIONS

- One should use appropriate normal neutrophil concentration values for certain ethnic groups in which neutrophil counts are significantly lower than persons of European ancestry (eg, African ancestry, Yemeni Jewish ancestry).
- In this classification, diseases resulting from neutrophil abnormalities in which the neutrophil is either the only cell type affected or is the dominant cell type affected are considered (**Table 29–1**).
- Neutropenia or neutrophilia occurs as part of disorders that affect multiple blood cell lineages (eg, aplastic anemia [see Chap. 3], myelodysplastic syndrome [see Chap. 44], acute and chronic myelogenous leukemias [see Chaps. 45 and 46], chronic myeloproliferative diseases [see Chaps. 41, 42, and 47]).
- A strict pathophysiologic classification of neutrophil disorders has proved elusive because:
  — The low concentration of blood neutrophils in neutropenic states makes measuring the circulatory kinetics of autologous cells technically difficult.
  — The two compartments of neutrophils in the blood, the random disappearance of neutrophils from the circulation, the extremely short circulation time of neutrophils ($t_{1/2}$ = ~6 hours), the absence of facile techniques to measure the size of the tissue neutrophil compartment, and the disappearance of neutrophils by apoptosis or gastrointestinal excretion from the tissue compartment make multicompartment kinetic analysis difficult.
- Thus, the classification of neutrophil disorders is partly pathophysiologic and partly descriptive (see Table 29–1).

TABLE 29–1 CLASSIFICATION OF NEUTROPHIL DISORDERS

I. Quantitative Disorders of Neutrophils
  A. Neutropenia
    1. Decreased neutrophilic granulopoiesis
      a. Congenital severe neutropenias (Kostmann syndrome and related disorders)
      b. Reticular dysgenesis (congenital aleukocytosis)
      c. Neutropenia and exocrine pancreas dysfunction (Shwachman-Diamond syndrome)
      d. Neutropenia and immunoglobulin abnormality (eg, hyperimmunoglobulin M syndrome)
      e. Neutropenia and disordered cellular immunity (cartilage hair hypoplasia)
      f. Mental retardation, anomalies, and neutropenia (Cohen syndrome)
      g. X-linked cardioskeletal myopathy and neutropenia (Barth syndrome)
      h. Myelokathexis
      i. Warts, hypogammaglobulinemia, infection, myelokathexis (WHIM) syndrome
      j. Neonatal neutropenia and maternal hypertension
      k. Griscelli syndrome

- l. Glycogen storage disease 1b
- m. Hermansky-Pudlak syndrome 2
- n. Wiskott-Aldrich syndrome
- o. Chronic hypoplastic neutropenia
  - (1) Drug-induced
  - (2) Cyclic
  - (3) Branched-chain aminoacidemia
- p. Acute hypoplastic neutropenia
  - (1) Drug-induced
  - (2) Infectious
- q. Chronic idiopathic neutropenia
  - (1) Benign
    - (a) Familial
    - (b) Sporadic
  - (2) Symptomatic

2. Accelerated neutrophil destruction
   - a. Alloimmune neonatal neutropenia
   - b. Autoimmune neutropenia
     - (1) Idiopathic
     - (2) Drug-induced
     - (3) Felty syndrome
     - (4) Systemic lupus erythematosus
     - (5) Other autoimmune diseases
     - (6) Complement activation-induced neutropenia
     - (7) Pure white cell aplasia
3. Maldistribution of neutrophils
   - a. Pseudoneutropenia

B. Neutrophilia

1. Increased neutrophilic granulopoiesis
   - a. Hereditary neutrophilia
   - b. Trisomy 13 or 18
   - c. Chronic idiopathic neutrophilia
     - (1) Asplenia
   - d. Neutrophilia or neutrophilic leukemoid reactions
     - (1) Inflammation
     - (2) Infection
     - (3) Acute hemolysis or acute hemorrhage
     - (4) Cancer, including granulocyte colony-stimulating factor (G-CSF)-secreting tumors
     - (5) Drugs (eg, glucocorticoids, lithium, granulocyte- or granulocyte-monocyte colony-stimulating factor, tumor necrosis factor-α)
     - (6) Ethylene glycol exposure
     - (7) Exercise
   - e. Sweet syndrome
   - f. Cigarette smoking
   - g. Cardiopulmonary bypass
2. Decreased neutrophil circulatory egress
   - a. Drugs (eg, glucocorticoids)
3. Maldistribution of neutrophils
   - a. Pseudoneutrophilia

II. Qualitative Disorders of Neutrophils

A. Defective adhesion of neutrophils

1. Leukocyte adhesion deficiency
2. Drug-induced

B. Defective locomotion and chemotaxis

1. Actin polymerization abnormalities
2. Neonatal neutrophils
3. Interleukin-2 administration
4. Cardiopulmonary bypass

C. Defective microbial killing

1. Chronic granulomatous disease

2. RAC-2 deficiency
3. Myeloperoxidase deficiency
4. Hyperimmunoglobulin E (Job) syndrome
5. Glucose-6-phosphate dehydrogenase deficiency
6. Extensive burns
7. Glycogen storage disease Ib
8. Ethanol toxicity
9. End-stage renal disease
10. Diabetes mellitus

D. Abnormal structure of the nucleus or of an organelle
1. Hereditary macropolycytes
2. Hereditary hypersegmentation
3. Specific granule deficiency
4. Pelger-Huët anomaly
5. Alder-Reilly anomaly
6. May-Hegglin anomaly
7. Chédiak-Higashi disease

III. Neutrophil-Induced Vascular or Tissue Damage
A. Pulmonary disease
B. Transfusion-related lung injury
C. Renal disease
D. Arterial occlusion
E. Venous occlusion
F. Myocardial infarction
G. Ventricular function
H. Stroke
I. Neoplasia
J. Sickle cell vasoocclusive crisis

RAC-2, RAS-related C3 botulinum toxin substrate 2.
Source: *Williams Hematology*, 9th ed, Chap. 64, Table 64–1.

NEUTROPENIA

- Certain childhood syndromes have been listed under decreased neutrophilic granulopoiesis. They could have been listed under chronic hypoplastic or chronic idiopathic neutropenia; however, they seem to hold a special interest as pediatric conditions and the causative gene mutations are known in many cases.
- Three childhood syndromes, although associated with neutropenia, are omitted because the neutropenia is part of a more global suppression of hematopoiesis: Pearson syndrome, Fanconi syndrome, and dyskeratosis congenita (see Chap. 3).
- Chronic idiopathic neutropenias include:
  — Cases with normocellular marrows but an inadequate compensatory increase in granulopoiesis for the degree of neutropenia (~1/3 of cases)
  — Cases with hyperplastic marrow granulopoiesis that is apparently ineffective (~1/2 of cases)
  — Cases with hypoplastic marrow granulopoiesis (~1/6 of cases)
- The clinical manifestations of decreased concentrations or abnormal function of neutrophils are the result of infection.
  — The relationship of frequency or type of infection to neutrophil concentration is an imperfect one.
  — The cause of the neutropenia, the coincidence of monocytopenia or lymphopenia,

concurrent use of alcohol or glucocorticoids, and other factors can influence the likelihood of infection.
  - — Infections in neutropenic persons not otherwise compromised are most likely to result, initially, from gram-positive cocci and usually are superficial, involving the skin, oropharynx, bronchi, anal canal, or vagina. However, any site may become infected, and gram-negative organisms, viruses, or opportunistic organisms may be involved.
  - — There is a decrease in the formation of pus in patients with severe neutropenia. This failure to suppurate can mislead the clinician and delay identification of the site of infection because minimal physical or radiographic findings develop.
  - — Exudate, swelling, and regional adenopathy are much less prevalent in severely neutropenic patients. Fever is common, and local pain, tenderness, and erythema are nearly always present despite a marked reduction in neutrophils.
- Some individuals may have apparent neutropenia because a larger fraction of their blood neutrophils is in the marginal rather than the circulating blood pool. In this type of neutropenia, the total blood neutrophil pool is normal, the neutrophil's ability to enter tissues is normal, and infections do not result from this atypical circulatory distribution of neutrophils. This type of alteration has been called *pseudoneutropenia*.

QUALITATIVE (FUNCTIONAL) NEUTROPHIL ABNORMALITIES

- Neutrophil function depends on the ability of neutrophils to adhere to vascular endothelium, penetrate endothelium, migrate along chemotactic gradients, and ingest and kill microorganisms on contact. Loss of any of these functions can predispose to infection (see Chap. 31).
- Defects in cytoplasmic contractile proteins, granule synthesis or contents, or intracellular enzymes may underlie a movement, ingestion, or killing defect.
- These defects may be inherited or acquired.
- Chronic granulomatous disease and Chédiak-Higashi disease are two examples of inherited disorders (see Chap. 31).
- Among the acquired disorders are those extrinsic to the cell, such as in the movement, chemotactic, or phagocytic defects of diabetes mellitus, alcohol abuse, and glucocorticoid excess.
- Acquired intrinsic disorders are usually manifestations of clonal myeloid diseases (eg, deficient granules in leukemic neutrophils) (see Chap. 44).
- Severe functional abnormalities in neutrophils can result in *Staphylococcus aureus, Klebsiella aerobacter, Escherichia coli*, and other catalase-positive microorganism infections (see Chap. 31).

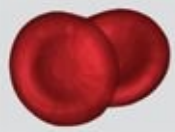
NEUTROPHILIA

- An overabundance of neutrophils has not been shown to result in specific clinical manifestations.
- Impairment of postischemic reperfusion of the coronary microcirculation has been thought to

be dependent, in part, on neutrophil plugging of myocardial capillaries.

NEUTROPHIL-INDUCED VASCULAR OR TISSUE DAMAGE

- Neutrophil products may contribute to the pathogenesis of inflammatory skin, bowel, synovial, glomerular, bronchial, retinal, and interstitial pulmonary diseases.
- Highly reactive oxygen products of neutrophils may be mutagens that increase the risk of neoplasia.
  — Development of carcinoma of the bowel in patients with chronic ulcerative colitis
  — Relationship between chronically elevated leukocyte count and the occurrence of lung cancer, independent of the effect of cigarette usage
- The oxidants, especially hypochlorous acid and chloramines, released by the neutrophils are extremely short-lived but may inactivate protease inhibitors in tissue fluids, permitting elastase, collagenase, gelatinase, and other proteases or cationic proteins to cause tissue injury.
- Expression of neutrophil selectins and integrin molecules (eg, intracellular adhesion molecule-1, endothelial-leukocyte adhesion molecule-1) contribute to neutrophils as pathogens. They contribute to microvascular damage by adherent neutrophils (eg, diabetic retinopathy; sickle cell vasculopathy [see Chap. 16]; transfusion-related acute lung injury [see Chap. 91]; specific types of renal, cerebral, retinal, and coronary vasculopathies; and other situations).
- Thrombogenesis has also been ascribed to leukocyte products, especially tissue factor.



For a more detailed discussion, see Marshall A. Lichtman: Classification and Clinical Manifestations of Neutrophil Disorders, Chap. 64 in *Williams Hematology*, 9th ed.

CHAPTER 30
Neutropenia and Neutrophilia

NEUTROPENIA

- *Leukopenia* refers to a reduced total leukocyte count.
- *Granulocytopenia* refers to a reduced granulocyte (neutrophils, eosinophils, and basophils) count.
- *Neutropenia* refers to a reduced neutrophil count: less than 1.5×10^9/L in patients from age 1 month to 10 years and less than 1.8×10^9/L in patients older than age 10 years. (**Table 29–1** outlines the classification of neutrophil disorders.)
- Agranulocytosis literally means a complete absence of blood granulocytes but is used to indicate very severe neutropenia, usually a neutrophil count less than 0.5×10^9/L.
- Americans of African descent (as do some other ethnic groups) have lower normal mean neutrophil counts than do Americans of European descent.
- The risk of infections is inversely related to the severity of the neutropenia: patients with qualitatively normal neutrophils and neutrophil counts of 1.0 to 1.8×10^9/L are at little risk, patients with counts of 0.5 to 1.0×10^9/L are at low or slight risk, and patients with counts less than 0.5×10^9/L are at higher risk.
- Patients with severe, prolonged neutropenia are at particular risk for bacterial and fungal infections.
- The risk is calculated not only by the neutrophil count but by complicating factors as follows:
  — The longer the duration of severe neutropenia, the greater the risk of infection.
  — The risk of infection is greater when the count is falling rapidly or when there is associated monocytopenia, lymphocytopenia, or hypogammaglobulinemia.
  — Neutropenia caused by disorders of hematopoietic progenitor cells (eg, chemotherapy-induced marrow suppression, severe inherited neutropenia) generally results in a greater susceptibility to infections compared with neutropenia resulting from accelerated turnover (eg, immune neutropenia).
  — Integrity of the skin and mucous membranes, blood supply to tissues, presence of an indwelling catheter, and nutritional status are also important in considering infection risk.
- Neutropenia can be classified as: (1) disorders of neutrophil production, (2) disorders of neutrophil distribution and turnover, (3) drug-induced neutropenia, and (4) neutropenia with infectious diseases.

DISORDERS OF PRODUCTION

Inherited Neutropenia Syndromes

Kostmann Syndrome

- Inheritance can be an autosomal dominant (mutation in gene for neutrophil elastase, *ELA-2*), recessive (mutation in gene encoding mitochondrial protein, *HAX-1*), or sporadic (mutation in *ELA-2*). Mutation in the gene for the glucose-6-phosphate catalytic subunit (*G6PC3*) also can cause severe neutropenia.
- Mutations in the receptor for granulocyte colony-stimulating factor (G-CSF) and in *RAS* may be present and, although not the cause of the neutropenia, may predispose to evolution to acute myelogenous leukemia.
- Otitis, gingivitis, pneumonia, enteritis, peritonitis, and bacteremia usually occur in the first month of life.
- Neutrophil count is often less than 0.2×10^9/L. Eosinophilia, monocytosis, and mild splenomegaly may be present.
- Marrow usually shows some early neutrophil precursors but few myelocytes or mature neutrophils.
- Immunoglobulin levels are usually normal or increased and chromosome analysis is normal.
- Treatment with G-CSF is usually effective in all types of hereditary neutropenia. It decreases recurrent fevers and infections. About 5% of patients do not respond.
- There is a risk of development of acute myelogenous leukemia.
- Allogeneic hematopoietic stem cell transplantation may be curative.

Neutropenia Associated with Congenital Immunodeficiency Diseases

- X-linked agammaglobulinemia, common variable immunodeficiency, and X-linked hyper-IgM syndrome are each accompanied by neutropenia in a proportion of patients.
- G-CSF may correct the neutropenia.
- Allogeneic hematopoietic stem cell transplantation may correct the primary immune disorder.
- Neutropenia is usually a production disorder based on marrow examinations showing decreased granulopoiesis.
- In X-linked agammaglobulinemia of Bruton (mutation in *BTK* gene), severe neutropenia is present in about 25% of patients.
- Children with common variable immunodeficiency commonly have neutropenia (and thrombocytopenia and hemolytic anemia).
- In X-linked hyperimmunoglobulin-M syndrome (mutation in gene encoding CD40 ligand), neutropenia is present in approximately 50% of patients.
- In severe combined immunodeficiency, neutropenia is a constant feature.
- Reticular dysgenesis results from thymic aplasia and inability to produce neutrophils or thymus- or marrow-derived lymphocytes. Neutropenia is severe, and patients have extreme susceptibility to bacterial and viral infections and often die at an early age.
- Allogeneic hematopoietic stem cell transplantation should be considered.

Cartilage-Hair Hypoplasia Syndrome

- This is a rare autosomal recessive disorder.
- Short-limbed dwarfism with hyperextensible digits and fine hair is apparent.
- Neutropenia, lymphopenia, and frequent infections occur.

- The marrow shows granulocytic hypoplasia.
- A defect in cellular immunity is present.
- Allogeneic hematopoietic stem cell transplantation can correct the hematopoietic and immune abnormality.

Shwachman-Diamond Syndrome

- Inheritance is autosomal recessive. Mutation in *SBDS* gene results in proliferative defect in and exaggerated apoptosis of hematopoietic precursors.
- The syndrome is characterized by short stature, pancreatic exocrine deficiency, steatorrhea, skeletal abnormalities, and developmental retardation.
- Neutropenia beginning in the neonatal period may be intermittent or cyclic and may be as low as 0.2×10^9/L. Anemia and thrombocytopenia occur in about one third of patients (see Chap. 3).
- Aplastic anemia or oligoblastic or acute myelogenous leukemia may develop (> 20% of patients who do not have successful allogeneic hematopoietic stem cell transplantation).
- Some patients may have an increase in neutrophil count with G-CSF.
- Allogeneic hematopoietic stem cell transplantation can correct the hematopoietic abnormality and markedly decrease the risk of transformation to acute myelogenous leukemia.

Chédiak-Higashi Syndrome

- This autosomal recessive disorder with oculocutaneous albinism results from mutation in *LYST* gene regulating lysosomal trafficking (see Chap. 31).
- Neutropenia is usually mild.
- Giant granules occur in granulocytes, monocytes, and lymphocytes.
- Recurrent infections result from moderate neutropenia and ineffective killing of microorganisms.

Failure of Neutrophil Marrow Egress

- Myelokathexis is a rare disorder associated with neutrophil counts of less than 0.5×10^9/L.
  — Marrow contains abundant myeloid precursors and mature neutrophils.
  — Marrow neutrophils show hypersegmentation, cytoplasmic vacuoles, and abnormal nuclei.
  — Neutrophil count does not rise with infection, suggesting that the primary disorder is neutrophil release from the marrow.
- Warts, hypogammaglobulinemia, infections, and myelokathexis (WHIM syndrome)
  — The syndrome is caused by mutation in *CXCR-4* gene (encodes the receptor for the stromal cell-derived factor 1) resulting in abnormal trafficking of cells out of marrow.
  — It may improve after G-CSF administration.
  — It can evolve into myelodysplastic syndrome or acute myelogenous leukemia.
- Lazy leukocyte syndrome
  — There are ample marrow precursors and neutrophils but few circulating cells. The neutrophils have a defect in intrinsic motility and do not migrate out of the marrow efficiently.

Glycogen Storage Disease Ib

- This condition is characterized by hypoglycemia, hepatosplenomegaly, seizures, and failure to thrive in infants. It is attributable to mutation in gene for intracellular transport protein for glucose.
- Severe neutropenia gradually develops despite normal-appearing marrow.
- Neutrophils have reduced oxidative burst and chemotaxis.
- Neutropenia may improve with G-CSF.
- It may evolve into acute myelogenous leukemia.

Cyclic Neutropenia

- Onset is usually in childhood.
- One third of patients have an autosomal dominant pattern of inheritance, with mutations in *ELA-2* gene.
- Recurring episodes of severe neutropenia, every 21 days, lasting 3 to 6 days, are characteristic.
- Malaise, fever, mucous membrane ulcers, and lymphadenopathy are found.
- This condition results from a defect in the regulation of hematopoietic stem cells.
- Diagnosis can be made by serial differential counts, at least three times per week for a minimum of 6 weeks.
- Cycling of other white cells, reticulocytes, and platelets may accompany neutrophil cycles.
- Most patients survive to adulthood, and symptoms are often milder after puberty.
- Fatal clostridial bacteremia has been reported.
- Careful observation is warranted with each neutropenic period.
- Treatment with G-CSF is effective. It does not abolish cycling but shortens the neutropenic periods sufficiently to lessen symptoms and infections.

Transcobalamin II Deficiency

- Neutropenia as an early feature of pancytopenia from vitamin B_{12} deficiency and megaloblastic hematopoiesis as a result of a deficiency in the cobalamin carrier. It is corrected by vitamin B_{12} treatment.

Neutropenia with Dysgranulopoiesis

- This condition is notable for ineffective granulopoiesis.
- Neutrophil precursors show abnormal granulation, vacuolization, autophagocytosis, and nuclear abnormalities.

Acquired Neutropenia Syndromes

Neutropenia in Neonates of Hypertensive Mothers

- Low-birth-weight infants with low neutrophil counts are common.
- Severe neutropenia and a high risk of infection may occur in the first several weeks postpartum.
- G-CSF may increase neutrophil count. Clinical benefit (decreased incidence of infection) has

not been documented.

Chronic Idiopathic Neutropenia

- This includes familial, severe or benign neutropenia, chronic benign neutropenia of childhood, and chronic idiopathic neutropenia in adults.
- Some patients with chronic neutropenia may have large granular lymphocyte leukemia (see Chap. 57).
- Patients have selective neutropenia and normal or near-normal red cell, reticulocyte, lymphocyte, monocyte, and platelet counts and immunoglobulin levels.
- The spleen size is normal or minimally enlarged.
- Marrow examination shows normal cellularity or selective neutrophilic hypoplasia; the ratio of immature to mature cells is increased, suggesting ineffective granulocytopoiesis.
- Clinical course can usually be predicted based on the degree of neutropenia, marrow examination, and prior history of fever and infections.
- Treatment with G-CSF will increase neutrophils in most patients, if symptomatic with recurrent infections.

Cytotoxic Drug Therapy

- This causes neutropenia by decreasing cell production, It is probably the most frequent cause of neutropenia in the United States.

Neutropenia as a Result of Diseases Causing Impaired Production

- Diseases affecting hematopoietic stem and progenitor cells, such as acute leukemia and aplastic anemia, are causal.

Neutropenia as a Consequence of Nutritional Deficiencies

- Neutropenia is an early and consistent feature of megaloblastic anemias due to vitamin B_{12} or folate deficiency.
- Copper deficiency can cause neutropenia in patients receiving total parenteral nutrition with inadequate supplies of trace metals, in patients who have had gastric resection, and in malnourished children.
- Mild neutropenia may occur in patients with anorexia nervosa.

Pure White Cell Aplasia

- This is a rare disorder exhibiting selective severe neutropenia.
- Marrow is devoid of neutrophils and their precursors.
- It is the counterpart to pure red cell aplasia.
- Such aplasia may be associated with thymoma or agammaglobulinemia.
- The presumptive mechanism is autoimmune.
- Treat with antithymocyte globulin, glucocorticoids, and/or cyclosporine.

DISORDERS OF NEUTROPHIL DISTRIBUTION AND TURNOVER

Alloimmune (Isoimmune) Neonatal Neutropenia

- This type of neutropenia is caused by the transplacental passage of maternal immunoglobulin G (IgG) antibodies against neutrophil-specific antigens inherited from the father.
- The disorder occurs in about 1 in 2000 neonates and usually lasts 2 to 4 months.
- Often it is not recognized until bacterial infections occur in an otherwise healthy infant, and it may be confused with neonatal sepsis.
- Hematologic picture usually consists of isolated severe neutropenia and marrow with normal cellularity but reduced numbers of mature neutrophils.
- Diagnosis is usually made with antineutrophil agglutination or immunofluorescence tests.
- Antibiotic treatment is used only when necessary; glucocorticoids should be avoided.
- Exchange transfusions to decrease antibody titers may be useful.

Autoimmune Neutropenia

Idiopathic Immune Neutropenia

- Neutrophil autoantibodies may accelerate neutrophil turnover and impair production.
- Patients usually have selective neutropenia and one or more positive tests for antineutrophil antibodies.
- It is difficult to distinguish cases of autoimmune neutropenia from chronic idiopathic neutropenia.
- Spontaneous remissions sometimes occur. Intravenous immunoglobulin is effective for some pediatric patients; response to glucocorticoids is unpredictable.

Systemic Lupus Erythematosus

- Neutropenia occurs in 50% of patients, anemia in 75% (direct antiglobulin test positive in one third), thrombocytopenia in 20%, and splenomegaly in 15%.
- Increased IgG on the surface of neutrophils, and marrow cellularity and maturation are normal.
- Neutropenia usually does not increase susceptibility to infections in the absence of treatment with glucocorticoids or cytotoxic drugs.

Rheumatoid Arthritis

- Less than 3% of patients with classic rheumatoid arthritis have leukopenia.

Sjögren Syndrome

- About 3% of patients have leukocyte counts of 2.0 to 5.0 $\times 10^9$/L, with a normal differential count. Severe neutropenia with recurrent infections is rare.
- Treatment of the neutropenia should be reserved for patients with recurrent infections.

Felty Syndrome

- Rheumatoid arthritis, splenomegaly, and leukopenia represent classic triad.

- Prominent neutropenia is a constant feature.
- Troublesome infections are common in patients with absolute neutrophil counts below 0.2×10^9/L.
- High levels of circulating immune complexes may play a role in neutropenia.
- Lymphopenia and a very high rheumatoid factor titer is seen. A subset of patients has large granular lymphocytic leukemia (see Chap. 58).
- No clear relationship exists between spleen size and neutrophil count.
- Two thirds of patients respond to splenectomy with an increase in neutrophil count, but two third of these responders relapse later.
- Splenectomy should be reserved for patients with severe, recurrent, or intractable infections.
- Improvement has been reported with lithium, gold, and methotrexate. Some clinicians favor weekly methotrexate therapy because of ease of administration, effectiveness, and comparatively lower toxicity.
- Rituximab and tocilizumab have been used, but response is unpredictable.
- Treatment with G-CSF or granulocyte-monocyte colony-stimulating factor (GM-CSF) may improve neutropenia but may exacerbate arthritic manifestations.
- Treatment of the neutropenia should be reserved for patients with recurrent infections.

Other Syndromes

- Sporadic reports occur in Hodgkin lymphoma, chronic autoimmune hepatitis, and Crohn disease.

Other Neutropenias Associated with Splenomegaly

- A variety of diseases may cause this type of neutropenia, including sarcoidosis, lymphoma, tuberculosis, malaria, kala-azar, and Gaucher disease, usually in association with thrombocytopenia and anemia.
- Neutropenia associated with splenomegaly may be due to immune mechanisms or sluggish blood flow through the spleen with trapping of neutrophils.
- The neutropenia is usually not of clinical significance and splenectomy is rarely indicated to correct neutropenia.

DRUG-INDUCED NEUTROPENIA

- Drugs may cause neutropenia because of (1) dose-related toxic effects or (2) by immune mechanisms.
- **Table 30–1** lists drugs implicated. Information about new drugs can be obtained from the manufacturer, a drug information center, or a poison control center.
- Dose-related toxicity refers to nonselective interference of the drug with protein synthesis or cell replication.
- Phenothiazines, antithyroid drugs, and chloramphenicol cause neutropenia by this mechanism.
- Dose-related toxicity is more likely to occur with multiple drugs, high plasma concentrations, slow metabolism, or renal impairment.
- Cases not dose-related may be allergic (ie, the immunologic mechanism is poorly understood

but appears to be similar to drug-induced hemolytic anemia). Neutropenia tends to occur relatively early in the course of treatment with drugs to which the patient has been previously exposed.

- Women, older persons, persons with history of allergies are more commonly affected with drug-induced neutropenia.
- Patients usually present with fever, myalgia, sore throat, and severe neutropenia.
- A high level of suspicion and careful clinical history are critical to identifying the offending drug.
- Differential diagnosis includes acute viral infections and acute bacterial sepsis.
- If other hematologic abnormalities are also present, hematologic diseases that cause bi- and tricytopenia should be considered.
- Once the offending drug is stopped, patients with sparse marrow neutrophils but normal-appearing precursor cells will have neutrophil recovery in 4 to 7 days. When early precursor cells are severely depleted, recovery may take considerably longer.
- Marrow biopsy soon after recovery may reveal a very large cohort of normal promyelocytes, simulating promyelocytic leukemia. Observation for 2 or 3 days establishes the normal recovery process.
- If febrile, cultures of throat, nasal cavities, blood and urine should be done and broad-spectrum antibiotics used.

TABLE 30–1 CLASSIFICATION OF WIDELY USED DRUGS ASSOCIATED WITH IDIOSYNCRATIC NEUTROPENIA

ANALGESICS AND ANTI-INFLAMMATORY AGENTS

- Indomethacin*
- Gold salts
- Pentazocine
- *Para*-aminophenol derivatives*
- Acetaminophen
- Phenacetin
- Pyrazolone derivatives*
- Aminopyrine
- Dipyrone
- Oxyphenbutazone
- Phenylbutazone

ANTIBIOTICS

- Cephalosporins
- Chloramphenicol*
- Clindamycin
- Gentamicin
- Isoniazid
- *Para*-aminosalicylic acid
- Penicillins and semisynthetic penicillins*
- Rifampin
- Streptomycin
- Sulfonamides*

Tetracyclines
Trimethoprim-sulfamethoxazole
Vancomycin

ANTICONVULSANTS

Carbamazepine
Mephenytoin
Phenytoin

ANTIDEPRESSANTS

Amitriptyline
Amoxapine
Desipramine
Doxepin
Imipramine

ANTIHISTAMINES—H_2 BLOCKERS

Cimetidine
Ranitidine

ANTIMALARIALS

Amodiaquine
Chloroquine
Dapsone
Pyrimethamine
Quinine

ANTITHYROID DRUGS*

Carbimazole
Methimazole
Propylthiouracil

CARDIOVASCULAR DRUGS

Captopril
Disopyramide
Hydralazine
Methyldopa
Procainamide
Propranolol
Quinidine
Tocainide

DIURETICS

Acetazolamide
Chlorthalidone
Chlorothiazide
Ethacrynic acid
Hydrochlorothiazide

HYPOGLYCEMIC AGENTS

Chlorpropamide
Tolbutamide

HYPNOTICS AND SEDATIVES

Chlordiazepoxide and other benzodiazepines

Meprobamate
PHENOTHIAZINES*
Chlorpromazine
Phenothiazines
OTHER DRUGS
Allopurinol
Clozapine
Levamisole
Penicillamine
Ticlopidine

*More frequently reported to cause neutropenia in epidemiologic studies.

Note: Documentation of the role of specific drugs in the causation of neutropenia is dependent on either (1) the frequency of the occurrence among patients, (2) the timing of the event in relationship to drug use, (3) the absence of alternative explanations, or (4) the inadvertent or intentional reuse of the drug (rechallenges) with a similar response. Readers who require supplementary lists of putative drugs involved in the development of neutropenia or wish to read original references for these interactions are referred to Table 65-1 in *Williams Hematology*, 9th ed.

Source: *Williams Hematology*, 9th ed, Chap. 65, Table 65–1.

NEUTROPENIA WITH INFECTIOUS DISEASES

- Such neutropenia occurs with acute or chronic bacterial, viral, parasitic, or rickettsial diseases.
- Some agents such as those causing infectious hepatitis, Kawasaki disease, and HIV infection may cause neutropenia and pancytopenia by infecting hematopoietic progenitor cells.
- In severe gram-negative bacterial infections, neutropenia is probably a result of increased adherence to the endothelium, as well as increased utilization of neutrophils at the site of infection. This mechanism may also occur in rickettsial infections and some viral infections.
- Chronic infections causing splenomegaly, such as tuberculosis, brucellosis, typhoid fever, and malaria, probably cause neutropenia by splenic sequestration and marrow suppression.

CLINICAL APPROACH TO THE PATIENT PRESENTING WITH NEUTROPENIA

- *Acute onset of severe neutropenia* often presents with fever, sore throat, and inflammation of the skin or mucous membranes. This is an urgent clinical situation requiring prompt cultures, intravenous fluids, and broad-spectrum antibiotics.
- In the absence of recent hospitalization and antibiotic exposure, infections are usually caused by organisms found on the skin, nasopharynx, and intestinal flora, and are sensitive to several antibiotics. Immediate evaluation should include a careful history with particular attention to drug use and physical examination with attention to skin; oronasopharynx; sinuses; lungs; lymph nodes; and abdomen, including liver and spleen size or bone tenderness.
- Prompt blood counts, microbial cultures, institution of intravenous fluids, and other supportive measures may be critical in an acute and severe situation. The history and physical examination may point to other needs such as chest or abdominal imaging.

- *Chronic neutropenia* is usually found by chance or during evaluation of recurrent fevers or infections. It is useful to determine whether the neutropenia is chronic or cyclic and the average neutrophil count when the patient is well.
- The absolute monocyte, lymphocyte, eosinophil, and platelet counts, as well as hemoglobin and immunoglobulin levels, should be determined, and the blood film should be studied carefully for reactive lymphocytes and abnormal cells.
- Marrow examination is useful if multilineage involvement suggests a clonal myeloid disease or another cause of multicytopenia (eg, aplastic anemia or megaloblastic anemia).
- It may be useful to measure antinuclear antibodies (ANA) and rheumatoid factor, or to obtain other serologic tests for collagen vascular diseases, especially if skin rashes or articular symptoms or signs are present.
- Examination of the blood and marrow may identify abnormal cells (eg, Chédiak-Higashi syndrome or large granular lymphocytic leukemia).
- Infectious and nutritional causes for chronic neutropenia are rare and seldom difficult to recognize.
- Measurements of ANA, in vitro marrow colony-forming activity, and studies of drug-induced neutropenia may require laboratory techniques available only in specialized laboratories.

NEUTROPHILIA

- Neutrophilia is an increase in the absolute neutrophil count (bands and mature neutrophils) to greater than 7.5×10^9/L.
- For infants younger than 1 month of age, the normal range is as high as 26×10^9/L.
- Extreme neutrophilia is often referred to as a *leukemoid reaction* because the height of the leukocyte count may simulate leukemia.
- Neutrophilia may occur as a result of:
  — An increase in neutrophil production, which is required for sustained neutrophilia
  — Accelerated release of neutrophil from the marrow "storage pool" into the blood
  — Shift from the marginal to circulating pool (demargination); (cannot generate more than a twofold to threefold increase in neutrophil count)
  — Reduced egress of neutrophils from the blood to tissues
  — A combination of these mechanisms
- The time required to develop neutrophilia may be:
  — Minutes (demargination)
  — Hours (accelerated release of neutrophils from marrow)
  — Days (increase in cell production)

ACUTE NEUTROPHILIA

- The causes are listed in **Table 30–2**.
- Pseudoneutrophilia is caused by a shift from the marginated to circulating pool (demargination) induced by vigorous exercise, by acute physical and emotional stress, or by the infusion of epinephrine.

- The marrow storage pool shifts involve the release of segmented neutrophils and bands from the marrow reserve in response to inflammation, infections, or colony-stimulating factors.

TABLE 30–2 MAJOR CAUSES OF NEUTROPHILIA

Acute Neutrophilia	Chronic Neutrophilia
Physical stimuli: cold, heat, exercise, convulsions, pain, labor, anesthesia, surgery Emotional stimuli: panic, rage, severe stress, depression Infections: many localized and systemic acute bacterial, mycotic, rickettsial, spirochetal, and certain viral infections Inflammation or tissue necrosis: burns, electric shock, trauma, infarction, gout, vasculitis, antigen–antibody complexes, complement activation Drugs, hormones, and toxins: colony-stimulating factors, epinephrine, etiocholanolone, endotoxin, glucocorticoids, smoking tobacco, vaccines, venoms	Infections: persistence of infections that cause acute neutrophilia Inflammation: most acute inflammatory reactions, such as colitis, dermatitis, drug-sensitivity reactions, gout, hepatitis, myositis, nephritis, pancreatitis, periodontitis, rheumatic fever, rheumatoid arthritis, vasculitis, thyroiditis, Sweet syndrome Tumors; gastric, bronchogenic, breast, renal, hepatic, pancreatic, uterine, and squamous cell cancers; rarely Hodgkin lymphoma, lymphoma, brain tumors, melanoma, and multiple myeloma Drugs, hormones, and toxins: continued exposure to many substances that produce acute neutrophilia, lithium; rarely as a reaction to other drugs Metabolic and endocrinologic disorders: eclampsia, thyroid storm, overproduction of adrenocorticotropic hormone Hematologic disorders: rebound from agranulocytosis or therapy of megaloblastic anemia, chronic hemolysis or hemorrhage, asplenia, myeloproliferative disorders, chronic idiopathic leukocytosis Hereditary and congenital disorders: Down syndrome, congenital

Source: *Williams Hematology*, 9th ed, Chap. 65, Table 65–2.

CHRONIC NEUTROPHILIA

- **Table 30–2** lists the causes of chronic neutrophilia.
- The neutrophil production rate increases up to threefold with chronic infections and even more in clonal myeloid disorders and in response to therapeutic administration of G-CSF or GM-CSF. The maximum response requires at least 7 to 10 days to develop.
- Neutrophilia as a result of decreased egress from the vascular compartment occurs with glucocorticoids, leukocyte adhesion deficiency (CD11/CD18 deficiency) (see Chap. 33), and recovery from infection.
- Chronic neutrophilic leukemia is a rare disorder with a very high blood concentration of neutrophils, often with few immature cells (see Chap. 46).

DISORDERS ASSOCIATED WITH NEUTROPHILIA

- Perhaps the most frequent are conditions with elevations of endogenous epinephrine and cortisol, such as exercise or emotional stress.
- The mean neutrophil count of people who smoke two packs of cigarettes daily is twice normal on average.
- Gram-negative infections, particularly those resulting in bacteremia or septic shock, may cause extreme neutrophilia or neutropenia.
- Some infections characteristically do not cause neutrophilia (eg, typhoid fever, brucellosis,

many viral infections).

- Neutrophilia in association with cancer may be a result of tumor cell secretion of colony-stimulating factors (especially G-CSF) or because of tumor necrosis and infection.
- In patients with cancer, subarachnoid hemorrhage, or coronary artery disease, neutrophilia may portend a less favorable prognosis.
- In addition to the clonal myeloid diseases, several unusual hematologic conditions may be associated with neutrophilia:
  — Hereditary disorders associated with thrombocytopenia may also be accompanied by leukemoid reactions (eg, thrombocytopenia with absent radii, see Chap. 75).
  — Benign idiopathic neutrophilic leukocytosis may be acquired or may occur as an autosomal dominant trait.
  — In Down syndrome, neonatal leukemoid reactions may resemble myelogenous leukemia.

Neutrophilia and Drugs

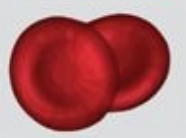
- Catecholamines and glucocorticoids are common causes of neutrophilia.
- Lithium causes neutrophilia, presumably because of G-CSF release.
- Rarely, other drugs will cause neutrophilia (eg, ranitidine or quinidine).

EVALUATION OF NEUTROPHILIA

- In most instances, the finding of neutrophilia, with an increase in bands and with toxic granules in the mature cells, can be related to an infection or inflammatory condition or, less commonly, the release of G-CSF by a neoplasm such as lung cancer.
- The history should make note of smoking, drug usage, or symptoms of occult malignancy.
- If the neutrophilia is accompanied by myelocytes, promyelocytes, increased basophils, and splenomegaly, a clonal myeloid disorder (eg, chronic myelogenous leukemia, primary myelofibrosis, chronic myelomonocytic leukemia) should be considered (see Chaps. 46 and 47).

THERAPY

- There are no direct adverse effects of an elevated neutrophil count in most situations in which the response is to an infection. Sickle cell crisis has been associated with chronic or recurring neutrophilia, as have some vasculopathies (see Chap. 29, “Neutrophil-Induced Vascular or Tissue Damage”). In the clonal myeloid diseases in which there are very elevated blast cell counts, adverse effects may occur (see Chap. 40, “Hyperleukocytic Syndromes”). The retinoic acid syndrome in acute promyelocytic leukemia treated with all-*trans* retinoic acid is a special situation in which morbid effects are often correlated with simultaneous neutrophilia (see Chap. 45).
- In some inflammatory diseases, glucocorticoids and immunosuppressive therapies are used to reduce inflammation in part by reducing production or altering distribution of neutrophils.
- Specific therapy, if indicated, is generally directed at the underlying cause of neutrophilia.



For a more detailed discussion, see Wayne C. Smith: Production, Distribution, and Fate of Neutrophils, Chap. 61; Marshall A. Lichtman: Classification and Clinical Manifestations of Neutrophil Disorders, Chap. 64; David C. Dale and Karl Welte: Neutropenia and Neutrophilia, Chap. 65 in *Williams Hematology*, 9th ed.

CHAPTER 31

Disorders of Neutrophil Functions

CATEGORIES OF NEUTROPHIL DYSFUNCTION

- Antibody or complement defects
- Abnormalities of cytoplasmic movement (chemotaxis and phagocytosis)
- Abnormal microbicidal activity
- See **Table 31–1** for features of the major abnormalities of neutrophil function

TABLE 31–1 CLINICAL DISORDERS OF NEUTROPHIL FUNCTION

Disorder	Etiology	Impaired Function	Clinical Consequence
Degranulation abnormalities			
Chédiak-Higashi syndrome	Autosomal recessive; disordered coalescence of lysosomal granules; responsible gene is *CHSI/LYST*, which encodes a protein hypothesized to regulate granule fusion	Decreased neutrophil chemotaxis; degranulation and bactericidal activity; platelet storage pool defect; impaired NK function, failure to disperse melanosomes	Neutropenia; recurrent pyogenic infections, propensity to develop marked hepatosplenomegaly as a manifestation of the hemophagocytic syndrome
Specific granule deficiency	Autosomal recessive; functional loss of myeloid transcription factor arising from a mutation or arising from reduced expression of Gfi-1 or C/eBpε, which regulates specific granule formation	Impaired chemotaxis and bactericidal activity; bilobed nuclei in neutrophils; defensins, gelatinase, collagenase, vitamin B_{12}-binding protein, and lactoferrin	Recurrent deep-seated abscesses
Adhesion abnormalities			
Leukocyte adhesion deficiency I	Autosomal recessive; absence of CD11/CD18 surface adhesive glycoproteins (β_2 integrins) on leukocyte membranes most commonly arising from failure to express CD18 mRNA	Decreased binding of C3bi to neutrophils and impaired adhesion to ICAM-1 and ICAM-2	Neutrophilia; recurrent bacterial infection associated with a lack of pus formation
Leukocyte adhesion deficiency II	Autosomal recessive; loss of fucosylation of ligands for selectins and other glycol conjugates arising from mutations of the GDP-fucose transporter	Decreased adhesion to activated endothelium expressing ELAM	Neutrophilia; recurrent bacterial infection without pus
Leukocyte adhesion deficiency III (LAD-I variant syndrome)	Autosomal recessive; impaired integrin function arising from mutations of *FERMT3*,	Impaired neutrophil adhesion and platelet activation	Recurrent infections, neutropenia, bleeding tendency

	which encodes kindlin-3 in hematopoietic cells; kindlin-3 binds to β-integrin and thereby transmits integrin activation		
Disorders of cell motility			
Enhanced motile responses; FMF	Autosomal recessive gene responsible for FMF on chromosome 16, which encodes for a protein called "pyrin"; pyrin regulates caspase-1 and thereby IL-1β secretion; mutated pyrin may lead to heightened sensitivity to endotoxin, excessive IL-1β production, and impaired monocyte apoptosis	Excessive accumulation of neutrophils at inflamed sites, which may be the result of excessive IL-1β production	Recurrent fever, peritonitis, pleuritis, arthritis, and amyloidosis
Depressed motile responses			
Defects in the generation of chemotactic signals	IgG deficiencies; C3 and properdin deficiency can arise from genetic or acquired abnormalities; mannose-binding protein deficiency predominantly in neonates	Deficiency of serum chemotaxis and opsonic activities	Recurrent pyogenic infections
Intrinsic defects of the neutrophil (eg, leukocyte adhesion deficiency, hédiak-Higashi syndrome, specific granule deficiency, neutrophil actin dysfunction, neonatal neutrophils); direct inhibition of neutrophil mobility (eg, drugs)	In the neonatal neutrophil there is diminished ability to express β_2 integrins and there is a qualitative impairment in β_2-integrin function; ethanol, glucocorticoids, cyclic AMP	Diminished chemotaxis; impaired locomotion and ingestion; impaired adherence	Propensity to develop pyogenic infections; possible cause for frequent infections; neutrophilia seen with epinephrine arises from cyclic AMP release from endothelium
Immune complexes	Bind to Fc receptors on neutrophils in patients with rheumatoid arthritis, systemic lupus erythematosus, and other inflammatory states	Impaired chemotaxis	Recurrent pyogenic infections
Hyperimmunoglobulin-E syndrome	Autosomal dominant; responsible gene is *STAT3*	Impaired chemotaxis at times; impaired regulation of cytokine production	Recurrent skin and sinopulmonary infections, eczema, mucocutaneous candidiasis, eosinophilia, retained primary teeth, minimal trauma fractures, scoliosis, and characteristic facies
Hyperimmunoglobulin-E syndrome	Autosomal recessive; more than one gene likely contributes to its etiology	High IgE levels, impaired lymphocyte activation to staphylococcal antigens	Recurrent pneumonia without pneumatoceles sepsis, enzyme, boils, mucocutaneous candidiasis, neurologic symptoms, eosinophilia
Microbicidal activity			
Chronic granulomatous disease	X-linked and autosomal	Failure to activate neutrophil	Recurrent pyogenic infections

	recessive; failure to express functional gp91^{phox} in the phagocyte membrane in p22^{phox} (autosomal recessive); other autosomal recessive forms of CGD arise from failure to express protein p47^{phox} or p67^{phox}	respiratory burst leading to failure to kill catalase-positive microbes	with catalase-positive microorganisms
G6PD deficiency	Less than 5% of normal activity of G6PD	Failure to activate NADPH-dependent oxidase, and hemolytic anemia	Infections with catalase-positive microorganisms
Myeloperoxidase deficiency	Autosomal recessive; failure to process modified precursor protein arising from missense mutation	H_2O_2-dependent antimicrobial activity not potentiated by myeloperoxidase	None
Rac-2 deficiency	Autosomal dominant; dominant negative inhibition by mutant protein of rac-2–mediated functions	Failure of membrane receptor–mediated O_2 generation and chemotaxis	Neutrophilia, recurrent bacterial infections
Deficiencies of glutathione reductase and glutathione synthetase	Autosomal recessive; failure to detoxify H_2O_2	Excessive formation of H_2O_2	Minimal problems with recurrent pyogenic infections

AMP, adenosine monophosphate; C, complement; CD, cluster designation; CGD, chronic granulomatous disease; ELAM, endothelial-leukocyte adhesion molecule; FMF, familial Mediterranean fever; G6PD, glucose-6-phosphate dehydrogenase; GDP, glucose diphosphate; ICaM, intracellular adhesion molecule; Ig, immunoglobulin; IL, interleukin; LAD, leukocyte adhesion deficiency; NADPH, nicotinamide adenine dinucleotide phosphate; NK, natural killer. Data from Remington JS, Swartz MN: *Current Clinical Topics in Infectious Disease*, 6th ed. New York: McGraw-Hill; 1985.

ANTIBODY/COMPLEMENT DEFECTS

- Interactions between antibodies and complement generate opsonins and stimulate chemotactic factor development.
- C3 deficiency (autosomal recessive inheritance) results in the most severe disorder.
- Homozygotes have no detectable C3 and suffer severe recurrent bacterial infections.
- Deficiency of other less centrally active complement proteins results in a milder condition.
- C3b inactivator or properdin deficiency results in deficiency of C3 also.
- Affected individuals usually suffer from infections due to encapsulated organisms.

GRANULE ABNORMALITIES

Chédiak-Higashi Syndrome

Pathogenesis

- This syndrome is a rare autosomal recessive disorder of abnormal, increased granule fusion with generalized cell dysfunction, resulting in defects in chemotaxis, degranulation, and microbicidal activity.
- The cause is mutation in *LYST* gene on chromosome 1q.
- There is increased membrane fluidity in Chédiak-Higashi neutrophils, monocytes, and natural

killer cells.
- Spontaneous fusion of granules results in huge lysosomes with diluted hydrolytic enzymes.
- Phagocytosis and the respiratory burst are normal, but killing of organisms is slow.
- Neutropenia occurs as a consequence of precursor death (apoptosis) in the marrow.

Clinical Features

- Because of abnormal association of melanosomes, decreased pigment is noted in skin, hair, iris, and ocular fundus. Light skin, silvery hair, solar sensitivity, and photophobia is characteristic.
- Total leukocyte counts average about 2×10^9/L, and neutrophil counts range from 0.5 to 2.0×10^9/L.
- Neutrophils and monocytes have impaired microbial killing as a result of inconsistent delivery of diluted granule contents into phagosome.
- Natural killer cell dysfunction may contribute to the predisposition to infection.
- Infections are common, primarily involving mucous membranes, skin, and the respiratory tract. Various bacteria and fungi are involved, but *Staphylococcus aureus* is the most common.
- Peripheral neuropathies (sensory and motor), cranial neuropathies, and autonomic dysfunction occur, as well as ataxia.
- Platelet counts are normal, but impaired platelet aggregation, storage pool deficiency, and prolonged closure times are common.
- An accelerated phase may occur at any age, characterized by rapid lymphocytic proliferation (not neoplastic) resulting in a syndrome of hepatosplenomegaly, lymphadenopathy, and high fever in the absence of bacterial infection. Subsequently, pancytopenia and a high susceptibility to infection usually lead to death. The syndrome is the result of an inherited predisposition to hemophagocytic lymphohistiocytosis, probably related to the inability to contain Epstein-Barr virus infection.

Laboratory Features

- In addition to the characteristic phenotypic features noted above, the principal confirmatory test is the presence of giant granules in neutrophils on the blood film. Molecular testing is not generally available. Heterozygotes are normal and cannot be detected clinically or biochemically.

Treatment

- High-dose ascorbic acid (200 mg/d in infants, 2 g/d in adults) is usually prescribed in the stable phase and improves the clinical state in occasional patients.
- Infections are treated as they arise. Prophylactic antibiotics are generally not useful.
- The only curative treatment is allogeneic hematopoietic stem cell transplantation from a histocompatible donor. The hematologic, immunologic, and natural killer cell abnormalities are corrected by successful transplantation. Other cell abnormalities are not.
- In the accelerated phase, vincristine and glucocorticoids have been used but are not clearly efficacious.

Specific Granule Deficiency

- This exceedingly rare autosomal recessive disorder is characterized by bilobed neutrophils lacking specific granules on the blood film and "empty" specific granules on transmission electron microscopy.
- Vitamin B_{12}-binding protein, lactoferrin, and collagenase are absent from specific granules. Defensins are absent from primary granules. Gelatinase activity is absent from tertiary granules. Microbicidal activity is moderately impaired because of a lack of defensins and lactoferrin.
- Chemotaxis is abnormal because of a lack of adhesion molecules in tertiary and specific granules.
- Eosinophil granule proteins are also deficient (major basic protein, eosinophilic cationic protein, eosinophil-derived neurotoxin). Thus, this disorder is a global abnormality of phagocyte granules and is not limited to neutrophil specific granules as its name implies.
- The diagnosis can be confirmed by a severe deficiency of lactoferrin or B_{12}-binding protein in the plasma.
- Recurrent skin and pulmonary infections are common, usually from infection with *S aureus* and *Pseudomonas aeruginosa. Candida albicans* infections also may occur.
- Treatment is supportive. Antibiotics for acute infections and surgical drainage of chronic abscesses.

ADHESION ABNORMALITIES

Leukocyte Adhesion Deficiency-I

- This rare, autosomal recessive disease is characterized by delayed detachment of umbilical cord, delayed wound healing, frequent severe periodontal or soft-tissue infection, and markedly decreased pus formation despite blood neutrophilia.
- The underlying defect is decreased or absent expression of the β_2 integrin family of leukocyte adhesion proteins (CD11/CD18 complex). These integral membrane glycoproteins (including LFA-1, Mac-1, and p150,95) have noncovalently bonded α and β subunits. Several mutations in the gene encoding the β subunit have been found; these mutations result in profoundly impaired chemotaxis or phagocytosis; degranulation and the respiratory burst are diminished. As a result, the neutrophils can enter the circulation but cannot egress into the tissues.
- See **Table 31–2** for features of leukocyte adhesion deficiency disorders.
- Blood neutrophil concentrations are markedly elevated (15–60 × 10^9/L), but cells do not enter tissues. Neutrophil concentrations may increase to as high as 150 × 10^9/L if patient is infected.
- Typically, there is marrow granulocytic hyperplasia and blood neutrophilia. Diagnosis is made by flow cytometric measurement of CD11a, CD11b, CD11c, and CD18 on neutrophils. Decreased expression of these surface molecules is the characteristic finding.
- Severely affected patients have recurrent and chronic soft-tissue infections (subcutaneous and mucous membranes). *S aureus*, *Pseudomonas* spp., other gram-negative enteric rods, and *Candida* spp. are the usual offenders.
- Prophylactic trimethoprim-sulfamethoxazole lowers the risk of recurrent infection.
- Treatment of choice for the severely affected is allogeneic hematopoietic stem cell

transplantation.

TABLE 31–2 BIOLOGIC AND CLINICAL FEATURES OF LEUKOCYTE ADHESION DEFICIENCIES I AND II

	Genetic Defect	Leukocyte Functional Abnormalities	Clinical Features	Diagnosis
LAD-I	Molecular mutations affecting expression of the β_2-integrin CD18	Neutrophils; adherence spreading, homotypic aggregation, chemotaxis receptor CR3 activities: C3bi binding affecting phagocytosis, respiratory burst, and degranulation in response to C3bi-coated particles* Monocytes; adherence, CR3 Activities Lymphocytes; cytotoxic T-lymphocyte activities; NK cytotoxic activities; blastogenesis	Autosomal recessive; delayed umbilical cord separation; neutrophilia; defective neutrophil migration into tissue; recurrent bacterial infections; impaired wound healing	Flow cytometry for expression of CD11b/CD18 (Mac-1)
LAD-II (CDG-IIc)	Mutations affecting function of GDP-fucose transporter 1 resulting in defective glycosylation expression at the α1, 3-position of selectin ligands including sLe^X and other fucosylated proteins requiring fucosylation	Neutrophils; rolling mediated by sLe^X to endothelium; neutrophilia†	Autosomal recessive; recurrent bacterial infections, periodontitis; growth retardation; developmental retardation; Bombay red cell phenotype	Flow cytometry for leukocyte sLe^X (CD15)

CDG-11c, congenital disorder of glycosylation type IIc; GDP, glucose diphosphate; NK, natural killer; sLe^X, sialyl Lewis X.
*These functional abnormalities and clinical features are a consequence of lack of the CD11b/CD18, which includes CD11a, CD11b, CD11c, and CD11d markers of four different α chains and the common β_2-chain CD18 of Mr 95 kDa.
†These functional abnormalities and clinical features are a consequence of lack of sLe^X expression on leukocytes.
Source: *Williams Hematology*, 9th ed, Chap. 66, Table 66–3.

MOVEMENT DISORDERS

Neutrophil Actin Dysfunction

- Abnormal chemotaxis and phagocytosis are expressed as neonatal recurrent severe bacterial infections.
- Defective actin polymerization occurs; an intracellular inhibitor of polymerization has been isolated.
- This rare lethal disease requires allogeneic hematopoietic stem cell transplantation.

Familial Mediterranean Fever

- This autosomal recessive disease primarily affects populations surrounding the Mediterranean basin. It is the result of a mutation in the *PYRIN* gene expressed primarily in leukocytes and synovial and peritoneal fibroblasts.

- The gene mutation may be identified by polymerase chain reaction methodology.
- The pathogenesis is a predisposition for neutrophils to migrate to serosal surfaces, accumulate, and generate an inflammatory response.
- The disease is characterized by acute limited attacks of fever, often accompanied by pleuritis; peritonitis; arthritis; pericarditis; inflammation of the tunica vaginalis of the testes; and erysipelas-like skin disease on the lower leg, ankle, or dorsum of the foot.
- Arthralgia and monarticular arthritis can accompany febrile attacks.
- Approximately 25% of affected patients develop renal amyloidosis. This finding can progress to renal failure and may be the cause of death.
- Prophylactic colchicine, 0.6 mg orally, two to three times a day, prevents or substantially reduces the acute attacks in most patients. Some patients, who have prodromes, can abort attacks with doses of colchicine beginning at the onset of attacks (0.6 mg orally every hour for 4 hours, then every 2 hours for four doses, and thereafter every 12 hours for 2 days).

Other Disorders of Neutrophil Motility

- Neonatal neutrophils have impaired β_2 integrin function with abnormal transendothelial movement.
- Direct inhibitors of neutrophil motility include ethanol and glucocorticoids.
- Circulating immune complexes also inhibit motility by binding to neutrophil Fc receptors.

Hyperimmunoglobulin E Syndrome

- This syndrome is usually an autosomal dominant disorder as a result of mutations in *STAT3* gene.
- Patients have markedly elevated serum IgE levels, chronic eczematoid dermatitis, and recurrent bacterial infections (skin abscesses, sinusitis, otitis media, pneumonia). They may also have coarse facial features, growth retardation, and osteoporosis.
- Coarse facial features include prominent forehead, broad nasal bridge, wide nasal tip, prognathism, hyperextensible joints, and scoliosis may be present. Delayed shedding of primary dentition occurs.
- Chemotaxis is impaired, but the molecular mechanism is unknown.
- Serum IgE levels usually exceed 2000 IU/mL, but, as opposed to atopic patients, most of this antibody is directed against *S aureus*.
- Marked blood and sputum eosinophilia are constant features. Poor antibody responses to neoantigens is also seen.
- Prophylactic trimethoprim-sulfamethoxazole is used to minimize frequency of *S aureus* infections.
- Early diagnosis and staphylococcal antibiotic prophylaxis can markedly improve the prognosis.
- Topical glucocorticoids may reduce symptoms of the eczematoid dermatitis.
- Orthopedic care for scoliosis, fractures, and degenerative joint disease, as well as dental care for delayed loss of first dentition are important.
- Incision and drainage of abscesses and superinfected pneumatoceles may be necessary.

DEFECTS IN MICROBICIDAL ACTIVITY

Chronic Granulomatous Disease (CGD)

- Neutrophils and monocytes have impaired production of superoxide, with markedly reduced microbicidal activity.
- CGD is caused by mutations in any of the genes encoding the NADPH oxidase, an electron transport chain that catalyzes the formation of superoxide.
- About two-thirds of patients inherit the neutrophil defect as an X chromosome–linked abnormality. The remaining patients have several types of autosomal inheritance.
- In the resting state, the oxidase components are in two locations. The membrane-bound portion, cytochrome b558, is composed of two subunits: gp91-*phox* and p22-*phox*. The heavy chain has binding sites for heme, FAD, and NADPH. Three other proteins reside in the cytosol, but on stimulation, move to the membrane and interact with gp91-*phox*. These are p47-*phox*, p67-*phox*, and a GTP-binding protein. Severity of CGD depends on which of these components is affected. The most frequent form is due to mutation of gp91-*phox* gene on chromosome Xp21.1. Other mutations also cause CGD but occur less frequently. See **Table 31–3** for the genetic and molecular classification of CGD.

TABLE 31–3 DIAGNOSTIC CLASSIFICATION OF CHRONIC GRANULOMATOUS DISEASE

Affected Component	Inheritance	Subtype	Membrane-Bound Cytochrome b_{558}*	Cytosol $p47^{phox}$*	Cytosol $p67^{phox}$*
$gp91^{phox}$	X	$X91^{0}$	Not detectable	Normal	Normal
		$X91^{+}$	Normal quantity, but nonfunctional	Normal	Normal
		$X91^{-}$	Defective gp 91^{phox}, which is poorly functional or expressed in a small fraction of phagocytes	Normal	Normal
$p22^{phox}$	A	$A22^{0}$	Not detectable	Normal	Normal
		$A22^{+}$	Normal quantity, but nonfunctional	Normal	Normal
$p47^{phox}$	A	$A47^{0}$	Normal quantity	Not detectable	Normal
$p67^{phox}$		$A67^{0}$	Normal	Normal	Not detectable

*Detected by spectral analysis or immunoblotting. In this nomenclature, the first letter represents the mode of inheritance (X-linked [X] or autosomal recessive [A]). The number indicates the phox component, which is genetically affected. The superscript symbols indicate whether the level of protein of the affected component is undetectable (0), diminished (–), or normal (+) as measured by immunoblot or spectral analysis.

Source: *Williams Hematology*, 9th ed, Chap. 66, Table 66–4.

Pathogenesis

- Normally, neutrophils form hydrogen peroxide, which acts as substrate for myeloperoxidase to oxidize chloride to hypochlorous acid and chloramines. These accumulate in the phagosome and kill the microbe.
- Oxidase activation acutely produces an alkaline phase in the phagosome that is important for

function of neutral hydrolases. In CGD cells, this alkaline phase does not occur, impairing the enzymes that digest bacteria.

Clinical Features

- The X-linked form can be evident in the first months of life, whereas autosomal forms may not be diagnosed until adulthood.
- Skin abscesses, recurrent lymphadenitis, dermatitis, pneumonias, osteomyelitis in small bones of hands or feet, and bacterial hepatic abscesses are each common and require consideration of chronic granulomatous disease. See **Table 31–4**.
- Organisms commonly involved are *S aureus*, *Aspergillus* sp, and *C albicans* (see **Table 31–4**).
- Granulomata are common and cause chronic lymphadenopathy.

TABLE 31–4 **COMMON INFECTING ORGANISMS ISOLATED FROM CHRONIC GRANULOMATOUS DISEASE PATIENTS**

Infection Type	Organism	X-Linked Recessive (%)	Autosomal Recessive (%)
Pneumonia	*Aspergillus* spp	41	29
	Staphylococcus spp	11	13
	Burkholderia cepacia	7	11
	Nocardia spp	6	13
	Serratia spp	4	5
Abscess			
Subcutaneous	*Staphylococcus* spp	28	21
	Serratia spp	19	9
	Aspergillus spp	7	0
Liver	*Staphylococcus* spp	52	52
	Serratia spp	6	4
	Candida spp	12	0
Lung	*Aspergillus* spp	27	18
Perirectal	*Staphylococcus* spp	9	15
Brain	*Aspergillus* spp	75	25
Suppurative adenitis	*Staphylococcus* spp	29	12
	Serratia spp	9	15
	Candida spp	7	4
Osteomyelitis	*Serratia* spp	32	12
	Aspergillus spp	25	18
Bacteremia/fungemia	*Salmonella* spp	20	13
	B cepacia	13	0
	Candida spp	9	25
	Staphylococcus spp	11	0

Source: *Williams Hematology*, 9th ed, Chap. 66, Table 66–5.

Laboratory Features

- For diagnosis, neutrophil superoxide or hydrogen peroxide generation is measured in response to soluble and particulate stimuli. Readout can be done using flow cytometry with dihydrorhodamine-123 label. The generation of hydrogen peroxide is used because it increases fluorescence on oxidation as an indicator of oxidative product formation.
- In addition, the nitroblue tetrazolium (NBT) test can be used. In normal neutrophils, NBT is reduced to purple formazan, and this assay is read by microscopic examination of individual neutrophils for purple formazan crystals. With most forms of CGD, no reduction to the purple color occurs.
- The NBT test can also detect the X-linked carrier state, because a varied percentage of cells will be NBT-negative and the remainder of the cells will be NBT-positive (mosaicism).
- More sophisticated procedures can define the molecular defect.
- Rare severe forms of glucose-6-phosphate dehydrogenase deficiency can mimic CGD; NADPH is inadequate for normal superoxide generation.

Therapy and Prognosis

- Treatment consists of long-term trimethoprim-sulfamethoxazole prophylaxis, appropriate antibiotics for particular infections, and surgical management of abscesses.
- Interferon-γ (50 $\mu g/m^2$, three times per week by subcutaneous injection) has been found to decrease the number of serious bacterial and fungal infections.
- The only curative therapy is allogeneic hematopoietic stem cell transplantation.
- Some affected persons and carriers who have only a small percent of normal functioning neutrophils have mild disease and a much better prognosis. This finding is most common in X chromosome–linked forms. Gene therapy is being studied, because one could predict that a very small increase in normally functioning neutrophils (eg, 5%) might significantly ameliorate the disease.

Myeloperoxidase Deficiency

- This common autosomal recessive disorder has a prevalence of 1:2000 in the general population.
- Myeloperoxidase (MPO) is absent in primary granules of neutrophils and monocytes (but not eosinophils).
- MPO catalyzes formation of hypochlorous acid; the MPO-deficient neutrophil is slower to kill ingested organisms, but after 1 hour, microbicidal activity is similar to normal as a result of MPO-independent killing systems in the cell.
- The disorder usually does not lead to increased susceptibility to infection.
- In a few patients with diabetes mellitus and MPO deficiency, severe infection with *Candida* sp has occurred.
- Acquired MPO deficiency can be seen in lead intoxication, myelodysplasia, acute myelogenous leukemia, and ceroid lipofuscinosis.

EVALUATION OF SUSPECTED NEUTROPHIL DYSFUNCTION

- Frequent bacterial infections should alert the clinician to the possibility of a functional neutrophil defect. Many of the tests used to evaluate neutrophils are bioassays, so are subject to great variability; they must be interpreted with caution, always in light of the patient's clinical condition. These tests are reviewed in **Figure 31–1**.

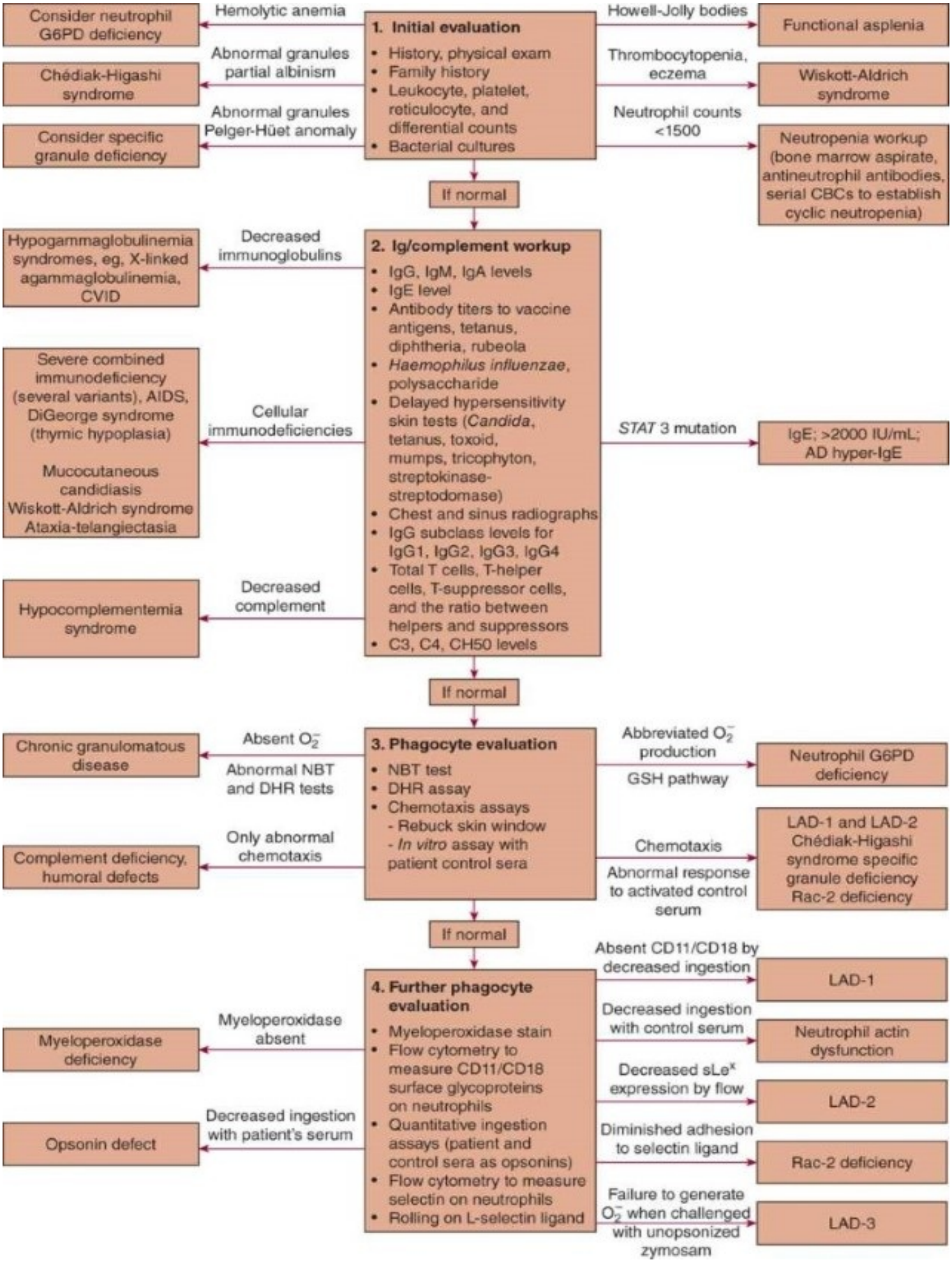
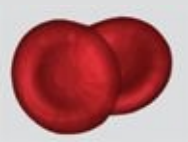

1. Initial evaluation
• History, physical exam
• Family history
• Leukocyte, platelet, reticulocyte, and differential counts
• Bacterial cultures
Hemolytic anemia
Consider neutrophil G6PD deficiency
Abnormal granules partial albinism
Chédiak-Higashi syndrome
Abnormal granules Pelger-Hüet anomaly
Consider specific granule deficiency
Howell-Jolly bodies
Functional asplenia
Thrombocytopenia, eczema
Wiskott-Aldrich syndrome
Neutrophil counts <1500
Neutropenia workup (bone marrow aspirate, antineutrophil antibodies, serial CBCs to establish cyclic neutropenia)
If normal
2. Ig/complement workup
• IgG, IgM, IgA levels
• IgE level
• Antibody titers to vaccine antigens, tetanus, diphtheria, rubeola
• Haemophilus influenzae, polysaccharide
• Delayed hypersensitivity skin tests (Candida, tetanus, toxoid, mumps, tricophyton, streptokinase-streptodomase)
• Chest and sinus radiographs
• IgG subclass levels for IgG1, IgG2, IgG3, IgG4
• Total T cells, T-helper cells, T-suppressor cells, and the ratio between helpers and suppressors
• C3, C4, CH50 levels
Decreased immunoglobulins
Hypogammaglobulinemia syndromes, eg, X-linked agammaglobulinemia, CVID
Cellular immunodeficiencies
Severe combined immunodeficiency (several variants), AIDS, DiGeorge syndrome (thymic hypoplasia)
Mucocutaneous candidiasis
Wiskott-Aldrich syndrome
Ataxia-telangiectasia
STAT 3 mutation
IgE; >2000 IU/mL; AD hyper-IgE
Decreased complement
Hypocomplementemia syndrome
If normal
3. Phagocyte evaluation
• NBT test
• DHR assay
• Chemotaxis assays
- Rebuck skin window
- In vitro assay with patient control sera
Absent O_2^-
Abnormal NBT and DHR tests
Chronic granulomatous disease
Only abnormal chemotaxis
Complement deficiency, humoral defects
Abbreviated O_2^- production
GSH pathway
Neutrophil G6PD deficiency
Chemotaxis
Abnormal response to activated control serum
LAD-1 and LAD-2 Chédiak-Higashi syndrome specific granule deficiency Rac-2 deficiency
If normal
4. Further phagocyte evaluation
• Myeloperoxidase stain
• Flow cytometry to measure CD11/CD18 surface glycoproteins on neutrophils
• Quantitative ingestion assays (patient and control sera as opsonins)
• Flow cytometry to measure selectin on neutrophils
• Rolling on L-selectin ligand
Myeloperoxidase absent
Myeloperoxidase deficiency
Decreased ingestion with patient's serum
Opsonin defect
Absent CD11/CD18 by decreased ingestion
LAD-1
Decreased ingestion with control serum
Neutrophil actin dysfunction
Decreased sLe^x expression by flow
LAD-2
Diminished adhesion to selectin ligand
Rac-2 deficiency
Failure to generate O_2^- when challenged with unopsonized zymosam
LAD-3

FIGURE 31–1 Algorithm for the evaluation of patients with recurrent infections. (Source: *Williams Hematology*, 9th ed, Chap. 66, Fig. 66–8.)



For a more detailed discussion, see Niels Borregaard: Disorders of Neutrophil Function, Chap. 66 in *Williams Hematology*, 9th ed.

CHAPTER 32
Eosinophils and Related Disorders

EOSINOPHIL PRODUCTION AND COUNTS

- Eosinophils differentiate from the hematopoietic stem cell in the marrow, migrate into the blood, and circulate with a half-life of 18 hours before entering tissues. Eosinophils are primarily tissue dwelling, with 300 cells in the tissues for every blood eosinophil.
- The normal blood absolute eosinophil count in adults is less than 0.4×10^9 cells/L; it is higher in neonates.
- The normal adult marrow contains approximately 3% eosinophils.

DEGREE AND CAUSES OF EOSINOPHILIA

- The degree of eosinophilia is described as:
  — Mild ($< 1.0 \times 10^9$ eosinophils/L)
  — Moderate (1.0 to 5.0×10^9 eosinophils/L)
  — Marked ($> 5.0 \times 10^9$ eosinophils/L)
- The most common causes of eosinophilia include:
  — Throughout the world: infections with helminthic parasites
  — In industrialized countries: asthma and other allergic disorders (drug allergy, allergic rhinitis, atopic dermatitis)
  — Allergic diseases: generally only mild eosinophilia
  — Major causes: listed in **Table 32–1**

TABLE 32–1 CAUSES OF EOSINOPHILIA

Disease	Frequency	Usual Degree of Eosinophilia	Comment
Infections			
Parasitic	Common worldwide	Moderate to high	
Bacterial	Rare		Usually cause eosinopenia, although serum ECP levels may be raised, suggesting eosinophil involvement in tissue.
Mycobacterial	Rare		More often secondary to drug therapy.
Invasive fungal	Unusual		Apart from allergic reactions, which are common, and coccidioidomycosis, in which as many as 88% of patients have an eosinophilia.
Rickettsial infections	Rare		

Fungi	Rare		Cryptococcus reported as causing CSF eosinophilia.
Viral infections	Rare		There are occasional case reports of an eosinophilia in a variety of viral infections, including herpes and HIV infection.
Allergic diseases			
Allergic rhinitis	Common worldwide	Mild	
Atopic dermatitis	Common especially children	Mild to moderate	
Urticaria/angioedema	Common	Variable	Eosinophilia seen in skin even with normal blood count.
Fungal allergy	Common	Mild to high	Immunoglobulin (Ig) E sensitization to thermotolerant colonizing yeast (eg, *Candida albicans*) and molds (eg, *Aspergillus fumigatus*) is a common cause of an eosinophilia.
Asthma	Common	Mild	Syndrome of intrinsic asthma, nasal polyps, and aspirin intolerance are associated with higher-than-usual eosinophil counts.
Drug reactions			
Many drugs	Uncommon	Mild to high	Antibiotics, NSAIDs, and antipsychotics are the most common groups; count usually returns to normal on stopping drug.
Neoplasms			
Acute eosinophilic leukemia	Rare	High	
Acute myelogenous leukemia with marrow eosinophilia	Uncommon	Mild to high in marrow only	Often associated with chromosome 16 abnormalities.
Chronic eosinophilic leukemia	Rare	High	See text on HES.
Chronic myelogenous leukemia	Uncommon	Moderate to high	Raised eosinophil counts can be seen uncommonly in chronic myelogenous leukemia.
Lymphomas	Uncommon	Moderate	Often intense tissue eosinophilia with moderately elevated blood eosinophil count; Hodgkin lymphoma most common type. T-cell lymphomas elaborating IL-5 or other eosinopoietic cytokines.
Langerhans cell histiocytosis	Uncommon	Mild	Intense tissue eosinophilia in granulomata but blood eosinophilia unusual.
Solid tumors	Uncommon	Mild to high	Many different tumors reported.
Musculoskeletal disorders			
Rheumatoid arthritis	Rare	Mild to high	Occasional case reports. More usually secondary to therapy.
Eosinophilic fasciitis	Rare	Moderate	
Gastrointestinal disorders			
Eosinophilic gastroenteritis	Rare	Mild to moderate	As with many GI diseases there is often a marked tissue eosinophilia with only a mild or no blood eosinophilia.
Eosinophilic esophagitis	Increasingly recognized	Mild	Marked tissue eosinophilia with mild or absent blood eosinophilia.

Celiac disease	Uncommon	None	Tissue eosinophilia.
Inflammatory bowel disease			Eosinophils seen in biopsies in both Crohn disease and ulcerative colitis, but blood eosinophilia unusual.
Allergic gastroenteritis	Rare	Mild to high	Young children.
Respiratory tract (for asthma, see Allergic diseases)			
Eosinophilic granulomatosis with polyangiitis	Rare	Moderate to high	Syndrome of eosinophilic vasculitis and asthma.
Chronic eosinophilic pneumonia	Uncommon	Mild to high	Syndrome of eosinophilia and chest x-ray shadowing
Bronchiectasis/cardia failure	Common	Mild	Often associated with asthma or allergic fungal airway disease.
Skin diseases (for atopic dermatitis, see Allergic diseases)			
Bullous pemphigoid	Uncommon	Moderate	
Eosinophilic cellulitis	Uncommon	Moderate to high	High eosinophil count distinguishes from bacterial cause.
Skin lymphoma (Sezary syndrome: mycosis fungoides)	Uncommon	Moderate	
Miscellaneous causes			
Interleukin (IL)-2 therapy	Rare	Moderate to high	For renal cell carcinoma or melanoma.
Hypereosinophilic syndrome	Rare	Moderate to High	
Endomyocardial fibrosis	Rare	High	Secondary to any cause of a high eosinophil count.
Hyper-IgE syndrome	Rare	Moderate to high	Possibly caused by fungal allergy.
Eosinophilia-myalgia and toxic oil syndrome	Rare Uncommon	High Mild to moderate	Two related conditions, one caused by poisoning with contaminated cooking oil in Spain and the other by a batch of contaminated tryptophan.
Graft-versus-host disease	Rare	Mild	
DOCK8 (dedicator of cytokinesis 8) deficiency	Rare		May be caused by fungal allergy
Olmstead syndrome	Rare		
Kimura disease	Uncommon		
Angiolymphoid hyperplasia			
Addison disease			

CSF, cerebrospinal fluid; ECP, eosinophil cationic protein; GI, gastrointestinal; HES, hypereosinophilic syndrome; NSAID, nonsteroidal anti-inflammatory drug.
Source: *Williams Hematology*, 9th ed, Chap. 62, Table 62–4.

EOSINOPHILS AND DISEASE

- Eosinophils appear to have a role in both ameliorating inflammatory responses and producing tissue damage.

- They may be important in wound healing and may act as accessory cells in T cell–mediated reactions.
- Because eosinophils can kill parasites, it has been hypothesized that their principal role is to counter parasitic infection.
- Eosinophils can cause airway damage in asthma and severe tissue damage in hypereosinophilic syndromes.

Asthma

- Inhaled antigen produces:
  - — An early response, with fall in forced expiratory volume caused by release of mediators from mast cells, may occur.
  - — A late response, with influx of large numbers of eosinophils, activated T cells, and monocytes to the airways, is also possible.
  - — Correlation exists between the numbers of airway eosinophils and the severity of asthma.
  - — Basic proteins secreted by eosinophils are toxic for airway epithelium and may be a better guide to the degree of inflammation than eosinophil numbers.

Parasitic Disease

- The mechanism of eosinophilia in parasitic disease is similar to allergic disease.
- The more pronounced eosinophilia in parasitic disease is presumably caused by the systemic nature of the disease compared with the more localized nature of allergic disease.
- Eosinophils can kill a number of opsonized parasite larvae but not adult worms.
- **Table 32–2** describes the major parasitic causes of eosinophilia.

TABLE 32–2 HELMINTHIC CAUSES OF EOSINOPHILIA

Parasite (Disease)	Comment
Nematodes	
Ascaris (Ascariasis)	Ascariasis results in higher eosinophil counts in children. Larvae migrate from intestine to lungs where they cause Loeffler syndrome, a form of pulmonary eosinophilia.
Toxocara canis (Toxocariasis)	Infective eggs are present in feces of puppies and pregnant bitches. Larvae in hosts such as chicken. Eosinophilia seen mainly in children younger than age 9 years. Can migrate to eye and cause blindness. Serologic evidence suggests infection not uncommon in industrialized countries.
Loa loa (Filariasis, Loiasis); *Wuchereria bancrofti* (Filariasis, Elephantiasis); *Brugia malayi* (Filariasis, Elephantiasis); *Onchocerca volvulus* (Filariasis, River Blindness)	Common. Invariably result in marked eosinophilia, especially *L loa* filariasis infection. Filariasis is the cause of tropical pulmonary eosinophilia caused by migration of adult worms to lung, elephantiasis caused by involvement of lymphatics (*W bancrofti* and *B malayi*) and river blindness (*O volvulus*). Treatment can result in systemic reaction called Mazzotti reaction, possibly as a result of massive eosinophil degranulation.
Ancylostoma duodenale (Ancylostomiasis, Old World Hookworm) and *Necator americanus* (New World Hookworm)	Hookworm infection. *A duodenale* and *N americanus*. One of the main causes of eosinophilia in patients returning from tropical countries. Eosinophil counts in region of $2000 \times 10^6/\mu L$.
Strongyloides stercoralis (Strongyloidiasis)	Subclinical infection can persist for more than 20 years. Stool examinations often negative. Cause of eosinophilia in ex-servicemen who spent time in tropics. If *Strongyloides* infection is not considered and these patients are given glucocorticoids for suspected hypereosinophilic syndrome or as trial of therapy,

	they can develop disseminated disease.
Trichinella spiralis (Trichinosis)	Trichinosis is caused by ingestion of encysted muscle larvae of *T spiralis*. Most prominent eosinophilia seen during early stages of infection when larvae migrating into striated muscle via the blood. Fatal cases reported of which only 20% were noted to have an eosinophilia.
Others	Other nematodes that can cause eosinophilia include *Trichuris trichiura* (Trichuriasis), *Capillaria philippinensis* (Capillariasis), and *Gnathostoma spinigerum* (Gnathostomiasis). The thread worm, *Enterobius vermicularis* (pinworm), occasionally causes an eosinophilia when they invade tissues.
Trematodes	
Schistosoma mansoni, S haematobium, S japonicum (Schistosomiasis, Bilharzia)	Infection with one of the *Schistosoma* (blood flukes), *S mansoni, S haematobium*, or *S japonicum*, is perhaps the most common cause of a moderate to high eosinophilia worldwide with 200 million people infected. Infection is nearly always associated with an eosinophilia.
Fasciola	Adult worms of *Fasciola hepatica* reside in the bile ducts, where they are associated with abnormal liver function tests and an eosinophilia.
Cestodes	
Echinococcus	Eosinophilia occurs in 25%–50% of patients with hydatid disease.

Source: *Williams Hematology*, 9th ed, Chap. 62, Table 62–5.

Idiopathic Hypereosinophilic Syndrome (Myeloid and Lymphoid Variants)

- This is a rare disorder with an estimated prevalence of one in 50,000. It occurs sporadically with no geographic or environmental influences.

Definition

- Striking eosinophilia ($> 1.5 \times 10^9$/L persists for more than 6 months, with evidence of end-organ damage and no other explanation after comprehensive investigation.

Myeloid Type

- Monoclonal eosinophilia reflects the expression of chronic eosinophilic leukemia. Marrow contains principally eosinophilic myelocytes and mature eosinophils, some with hypersegmented nuclei (more than two segments).
  - — Onset occurs with some or all of the following: anorexia, weight loss, fatigue, nausea, abdominal pain, diarrhea, nonproductive cough, pruritic rash, fever, night sweats, and venous thrombosis.
  - — Skin signs include urticaria, papules, and pruritus.
  - — Cardiac involvement may be marked by endomyocardial fibrosis, restrictive cardiomyopathy, mitral or tricuspid valve incompetence, ventricular failure, conduction defects, and arrhythmias.
  - — Neurologic findings include neuropathies; these may lead to encephalopathy, polyneuropathy, or stroke.
  - — Hepatosplenomegaly, interstitial pulmonary infiltrates, and pleural effusions can occur.
  - — Nervous system dysfunction may be profound, including confusion, delirium, dementia, and coma.

— Hematologic manifestations. All patients have leukocytosis with a striking eosinophilia, usually eosinophil counts greater than 1.5×10^9/L, and counts of 50.0×10^9/L or more are found in more than half the patients; eosinophilia may be progressive; anemia occurs in most patients; and thrombocytopenia is seen occasionally.
— Cytogenetic abnormalities may be found in the leukemic eosinophilic cells in a proportion of patients. One of several translocations involving chromosome 5 may occur. Of particular importance is the occurrence of the *FILIPI-PDGFR*-α fusion gene because, if present, the patient will usually respond to tyrosine kinase inhibitor drugs, such as imatinib mesylate.
— Elevated serum tryptase is common (suggesting unapparent mast cell involvement).
— The disease is sometimes indolent but more often progressive and fatal.
— Signs and symptoms may remit and relapse, but organ damage is usually progressive.
— Episodes of venous thrombosis may complicate the course.
— Therapy. In patients unresponsive to tyrosine kinase inhibitors, hydroxyurea and glucocorticoids may be of benefit in decreasing the eosinophil count and the risk of tissue injury. Other therapies include etoposide, interferon-α, cladribine, and leukapheresis. Allogeneic hematopoietic cell transplantation has been used in some patients.
— Surgical replacement of severely damaged heart valves has been successful.

Lymphoid Type

- Polyclonal eosinophilia may be associated with a clonal T-lymphocyte disorder (lymphoma), with elaboration of eosinopoietic cytokines, especially interleukin-5. It is less common than the myeloid type.
  — Eosinophil-induced tissue damage is not a feature of the disease.
  — Therapy. Treatment is directed principally at the underlying lymphoma.

Other Types

- The causes of the remaining cases of hypereosinophilia that are neither chronic eosinophilic leukemia nor related to lymphoma are still unclear.

Eosinophilia–Myalgia Syndrome

- This syndrome was first described in 1989, with more than 1500 cases reported and 30 deaths in the next 2 years.
- It is caused by the ingestion of L-tryptophan, a nutritional supplement, containing a contaminant.
- Constant features are severe myalgia and eosinophil count greater than 1.0×10^9/L.
- Common findings are arthralgias, cough, dyspnea, edema, hair loss, peripheral neuropathy, and scleroderma-like skin changes.
- Pathologic features mimic eosinophilic fasciitis (see below).
- Glucocorticoids have improved symptoms and signs.

Toxic Oil Syndrome

- In 1981, in Spain, more than 20,000 people developed a syndrome of fever, cough, dyspnea,

neutrophilia, and eosinophilia. More than 300 deaths occurred.
- It is thought to be caused by ingestion of aniline-denatured rapeseed oil.
- Pulmonary infiltrates, pleural effusion, and hypoxemia were common findings.
- One-half the patients developed a chronic illness that mimicked the eosinophilia–myalgia syndrome (see above), with myalgias, eosinophilia, peripheral neuritis, scleroderma-like skin lesions, hair loss, and sicca syndrome.
- Glucocorticoids may have improved the pulmonary symptoms.

Reactive Hypereosinophilia and Neoplasms

- Marked eosinophilia has been reported in association with a variety of solid tumors and lymphomas (eg, Hodgkin lymphoma), believed to be caused by interleukin-5 and other cytokines elaborated by tumor cells.
- Eosinophilia usually occurs concomitantly with the clinical diagnosis of the tumor.
- Successful treatment of the malignancy may be associated with amelioration of eosinophilia.
- Unlike other types of reactive eosinophilia, the blood eosinophil count may not be suppressed by glucocorticoids.

Acute Eosinophilic Leukemia

- This is a rare form of acute myelogenous leukemia.

Eosinophilic Granulomatosis with Polyangiitis

- This rare disorder of unknown etiology is characterized by eosinophilic asthma, chronic rhinosinusitis, and small vessel vasculitis.
- Forty percent of patients are antineutrophil cytoplasmic antibody (ANCA)–positive, and these patients have more vasculitic and renal disease manifestations, but less cardiac disease, than ANCA-negative patients (although there is considerable overlap).
- Diagnostic hallmark is eosinophilic vasculitis with granuloma on biopsy.
- Most patients enter remission with high-dose oral glucocorticoids, although guidelines recommend use of other immunosuppressants, such as cyclophosphamide or azathioprine, to induce remission where there is evidence of life-threatening organ involvement.

Eosinophilic Fasciitis

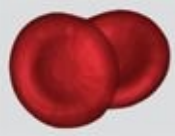
- This rare syndrome is characterized by stiffness, pain, and swelling of the arms, forearms, thighs, legs, hands, and feet in descending order of frequency.
- Malaise, fever, weakness, and weight loss also occur.
- Absolute eosinophil counts of greater than 1.0×10^9/L are found in most patients but may be intermittent.
- Biopsy is usually required for diagnosis and shows inflammation, edema, thickening, and fibrosis of the fascia and synovia.
- Aplastic anemia, cytopenias, pernicious anemia, and myelogenous leukemia have been associated.

EOSINOPHILS IN URINE AND CEREBROSPINAL FLUID

- Excretion of eosinophils in the urine is seen most often in urinary tract infection or acute interstitial nephritis.
- Cerebrospinal fluid eosinophilia may occur with infection, shunts, allergic reactions involving the meninges, and Hodgkin lymphoma.

EOSINOPENIA

- The eosinophil count in hospitalized patients is less than 0.01×10^9/L in only 0.1% of patients, often ascribed to glucocorticoids, acute infection or disease activity.



For a more detailed discussion, see Andrew J. Wardlaw: Eosinophils and Related Disorders, Chap. 62 in *Williams Hematology*, 9th ed.

CHAPTER 33
Basophils, Mast Cells, and Related Disorders

BASOPHILS

- The basophil is the least common granulocyte.
- Normal basophil count is 0.015 to 0.08 × 10^9/L.
- The causes of basopenia (decreased numbers) and basophilia (increased numbers) are listed in Table 33–1.

TABLE 33–1 CONDITIONS ASSOCIATED WITH ALTERATIONS IN NUMBERS OF BASOPHILS

I. Decreased Numbers (Basopenia)
- A. Hereditary absence of basophils (very rare)
- B. Elevated levels of glucocorticoids
- C. Hyperthyroidism or treatment with thyroid hormones
- D. Ovulation
- E. Hypersensitivity reactions
  1. Urticaria
  2. Anaphylaxis
  3. Drug-induced reactions
- F. Leukocytosis (in association with diverse disorders)

II. Increased Numbers (Basophilia)
- A. Allergy or inflammation
- B. Ulcerative colitis
- C. Drug, food, inhalant hypersensitivity
- D. Erythroderma, urticaria
- E. Juvenile rheumatoid arthritis
- F. Endocrinopathy
  1. Diabetes mellitus
  2. Estrogen administration
  3. Hypothyroidism (myxedema)
- G. Infection
  1. Chickenpox
  2. Influenza
  3. Smallpox
  4. Tuberculosis
- H. Iron deficiency
- I. Exposure to ionizing radiation
- J. Neoplasia
  1. "Basophilic leukemias" (see text)
  2. Myeloproliferative neoplasms (especially chronic myelogenous leukemia; also polycythemia vera, primary myelofibrosis, essential thrombocythemia)
  3. Carcinoma

Source: *Williams Hematology*, 9th ed, Chap. 63, Table 63–2.

BASOPENIA

- Hereditary absence of basophils is very rare.
- Other causes include high doses of glucocorticoids, hyperthyroidism or therapy with thyroid hormones, ovulation, hypersensitivity reactions, or leukocytosis in association with diverse disorders.

BASOPHILIA

- Causes include allergy or inflammation, endocrinopathies (diabetes mellitus, hypothyroidism), infections, iron deficiency, exposure to ionizing radiation, and neoplasias.
- Basophilia occurs in virtually all patients with chronic myelogenous leukemia (CML).
- De novo acute basophilic leukemia is very rare, but marrow basophilia may be associated uncommonly with other subtypes of acute myelogenous or acute promyelocytic leukemia.
- Basophils in acute or chronic clonal myeloid diseases are derived from the malignant clone and occasionally may cause symptoms of histamine release (flushing, pruritus, hypotension) or severe peptic ulcer disease reflecting hypersecretion of gastric acid and pepsin.

MAST CELLS AND SECONDARY CHANGES IN NUMBERS

- Mast cells are produced in the marrow and then transit the blood to the tissues where they reside. They cannot be identified in transit in the blood of healthy individuals by standard techniques.
- Mast cells contain mediators that may be preformed in granules (eg, histamine, heparin, and chemotactic factors) or newly formed (eg, arachidonic acid metabolites, such as prostaglandin D_2 and leukotrienes).
- An increased number of mast cells may be seen in tissues of immunoglobulin E–associated disorders, connective tissue disorders, at infection sites, and in the lymph nodes and marrow in a variety of benign and malignant tumors (see **Table 33–2**).

TABLE 33–2 CONDITIONS ASSOCIATED WITH SECONDARY CHANGES IN MAST CELL NUMBERS

I. Decreased Numbers
  A. Long-term treatment with glucocorticoids
  B. Primary or acquired immunodeficiency disorders (certain mast cell populations)

II. Increased Numbers
  A. IgE-associated disorders
    1. Allergic rhinitis
    2. Asthma
    3. Urticaria
  B. Connective tissue disorders
    1. Rheumatoid arthritis
    2. Psoriatic arthritis
    3. Scleroderma
    4. Systemic lupus erythematosus
  C. Infectious diseases
    1. Tuberculosis
    2. Syphilis

3. Parasitic diseases
D. Neoplastic disorders
1. Lymphoproliferative diseases* (lymphoplasmacytic lymphoma/Waldenström macroglobulinemia, other lymphomas, chronic lymphocytic leukemia)
2. Hematopoietic multipotential progenitor cell diseases* (acute or chronic myelogenous leukemias, myelodysplastic syndromes)
E. Lymph nodes draining areas of tumor growth
F. Osteoporosis*
G. Chronic liver disease*
H. Chronic renal disease*

*Indicates increase numbers of mast cells may be principally in marrow.
Source: *Williams Hematology*, 9th ed, Chap. 63, Table 63–4.

SYSTEMIC MASTOCYTOSIS

- This encompasses a group of systemic disorders associated with significant increases in mast cell numbers in the skin and internal organs.
- A consensus classification has been developed to provide prognosis and treatment (see **Table 33–3**).

TABLE 33–3 WORLD HEALTH ORGANIZATION CLASSIFICATION OF SYSTEMIC MASTOCYTOSIS

I. Cutaneous mastocytosis (CM) A. Urticaria pigmentosa (UP)/Maculopapular cutaneous mastocytosis (MPCM) B. Diffuse cutaneous mastocytosis C. Solitary mastocytoma of skin
II. Indolent systemic mastocytosis (ISM)
III. Systemic mastocytosis with associated clonal, hematologic non–mast-cell lineage disease (SM-AHNMD)
IV. Aggressive systemic mastocytosis (ASM)
V. Mast cell leukemia (MCL)
VI. Mast cell sarcoma (MCS)
VII. Extracutaneous mastocytoma

Modified with permission from Swerdlow SH, Campo E, Harris NL: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th ed. Lyon: IARC Press; 2008.

Clinical Features

- The clinical pattern and prognosis vary substantially among patients.
- Half the patients are older than 60 years of age at the time of diagnosis.
- Malaise, weight loss, and fever are frequent.
- Symptoms of mediator release include urticaria, pruritus, dermatographism, abdominal cramps, diarrhea, nausea, vomiting, musculoskeletal pain, flushing, headaches, dizziness, palpitations, dyspnea, hypotension, syncope, and shock.

Organ Involvement

- The organs most frequently involved are the skin, lymph nodes, liver, spleen, marrow, and gastrointestinal tract.

- Skin involvement is typically characterized by urticaria pigmentosa (UP) and is diagnosed before age 2 years in 50% of cases. Dermal accumulations of mast cells results in brown papules symmetrically distributed, especially over the trunk. Intense pruritus and urticaria may occur from mild friction of the skin (Darier sign). It typically subsides at puberty but can continue into adulthood (see **Figure 33–1**). Adults with UP usually have extracutaneous involvement by mastocytosis.
- Lymphadenopathy, hepatomegaly, splenomegaly, and bone pain are frequently present, especially in aggressive disease.
- The majority of adults have focal mast cell lesions in the marrow, but this is much less common in children.
- Osteoporosis may accompany systemic disease, and pathologic fractures may occur.

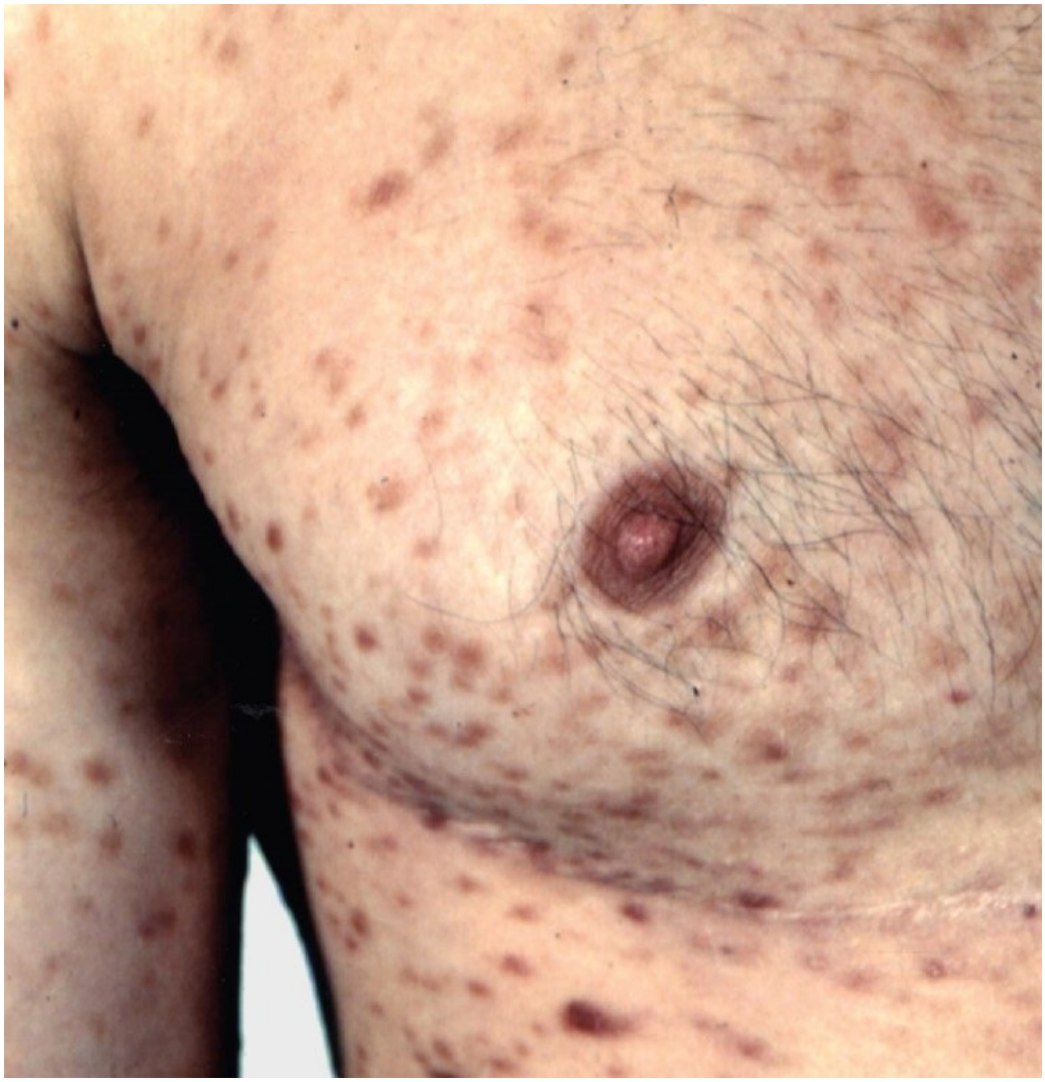


FIGURE 33–1 Urticaria pigmentosa in an adult man with indolent systemic mastocytosis. Multiple pigmented macules are present. If local pressure is applied to the skin, individual lesions show urtication and become raised, pruritic, and erythematous. (Source: *Williams Hematology*, 8th ed, Chap. 63, Fig. 63–2, p. 924.)

Laboratory Features

- Anemia is present in about 50% of cases at the time of diagnosis.
- Marrow biopsy shows an increase in mast cells in about 90% of patients. Immunohistochemical staining for mast cell tryptase is best for visualizing and quantifying

mast cell involvement.

- Mast cells in paraffin tissues are strongly positive for CD117, as are a subset of leukemic myeloblasts. Mast cells, unlike the latter, are not positive for peroxidase, however.
- Mast cells in the blood indicate a transformation to leukemia.
- Elevated alkaline phosphatase, aminotransaminases, and γ-glutamyltranspeptidase reflect liver involvement in about 50% of patients.
- Skin biopsy shows mast cell accumulations.
- Osteoporosis, osteoblastic, or osteolytic lesions are common on bone imaging studies.
- Aberrant mast cell phenotype by flow cytometry: high side scatter cells (granular) with surface IgE, and CD2+, CD25+, CD35+, CD117+, and CD34– immunophenotype.
- Finding of elevated plasma histamine levels and urinary excretion of the histamine metabolite 1-methyl-4-imidazoleacetic acid are useful diagnostic tests.
- Elevated mast cell tryptase in the serum is an important confirmatory finding.
- Elevated serum or urine histamine or serum tryptase is not pathognomonic of mastocytosis, however, and needs to be integrated with the clinical findings.
- Gain-of-function *KIT* gene mutation, Asp816Val, is a virtually universal finding in adults and many children with mastocytosis.
- **Table 33–4** lists the diagnostic criteria for systemic mastocytosis.

TABLE 33–4 DIAGNOSTIC CRITERIA FOR SYSTEMIC MASTOCYTOSIS

I. Major Criteria
  A. Multifocal, dense infiltrates of mast cells (≥15 mast cells in an aggregate) detected in sections of marrow and/or other extracutaneous organ(s)

II. Minor Criteria
  A. In biopsy sections of marrow or other extracutaneous organs, > 25% of the mast cells in the infiltrate are spindle shaped or have atypical morphology, or, of all mast cells in marrow aspirate smears, > 25% are immature or atypical mast cells
  B. Detection of a point mutation in *KIT* at codon 816 (Asp816V) in marrow, blood, or other extracutaneous organ
  C. Mast cells in marrow, blood, or other extracutaneous organs that co-express CD117 with CD2 and/or CD25
  D. Serum total tryptase persistently > 20 µg/L (if there is an associated clonal myeloid disorder, this criterion is not valid)

The diagnosis of systemic mastocytosis can be made if one major and one minor criterion are present or if three minor criteria are met.

Source: *Williams Hematology*, 9th ed, Chap. 63, Table 63–6.

Treatment

- Mastocytosis currently has no curative therapy. Symptomatic therapy, although transiently helpful, does not alter course of the disease.
- Local lesions may be excised.
- Avoid triggers such as temperature extremes; physical exertion; or in some cases, opiate analgesics, nonsteroidal anti-inflammatory drugs, and ingestion of ethanol-containing drinks.
- Anaphylaxis may follow insect stings. Epinephrine-filled syringes and instructions for self-administration should be carried by patients considered at risk. These patients may also benefit from prophylactic antihistamines in settings and during seasons in which insect stings are prevalent. Patients with mastocytosis can also have anaphylaxis from iodinated contrast material.
- Cutaneous glucocorticoids and 8-methoxypsoralen and ultraviolet light (PUVA) have been reported to decrease pruritus or improve the appearance of skin lesions.

- Histamine-2 (H_2)-receptor antagonists (eg, cimetidine, ranitidine, famotidine) can decrease gastric hyperacidity and can be used to treat gastritis or peptic ulcer. Proton pump inhibitors may also be useful in treating gastric hypersecretion. They may, in combination with histamine-1 (H_1)-receptor antagonists, contribute to ameliorating mast cell constituent release–related signs and symptoms.
- H_1-receptor antagonists (eg, diphenhydramine, chlorpheniramine, tricyclic antidepressants) can decrease flushing, vasodilation, and headache. More potent H_1-receptor blockers (hydroxyzine and doxepin) may be useful in more severe cases.
- Oral disodium cromoglycate may alleviate gastrointestinal cramping, diarrhea, and headache. It has also been useful in childhood cutaneous mast cell disease.
- Calcium supplementation, estrogen replacement in postmenopausal women, and bisphosphonates may be used to prevent/treat underlying osteopenia/osteoporosis.
- Oral glucocorticoids can be used for malabsorption or ascites. In adults, the doses used to start therapy are approximately 40 to 60 mg/d for 2 to 3 weeks and then they are tapered, eventuating in alternate-day use, if they are helpful.
- Insufficient data are available to determine the usefulness of cytotoxic agents, such as cladribine, for progressive mastocytosis. Chemotherapy, generally, has been disappointing in cases of aggressive systemic disease.
- Allogeneic stem cell transplantation has been used for patients with advanced categories of mastocytosis; however, although the associated hematologic disorder may respond, complete remission of mast cell disease is uncommon.
- Tyrosine kinase inhibitors may be useful. Rare mutations such as *KIT* (Phe522Cys) are responsive to imatinib. Tyrosine kinase inhibitors, including midostaurin and dasatinib, that inhibit the most prevalent *KIT* mutation at codon 816 (Asp816Val) are in clinical trials. If possible, mutational analysis on the mastocytosis cells should be done to determine if a drug-sensitive mutated kinase is present.
- In all the therapy noted above, success is unpredictable, the treatment has its own consequential side effects, and one must exercise judgment continually about whether the treatment is resulting in a net benefit to the patient.

Course and Prognosis

- Symptoms range from absent to progressive and disabling. Patients with UP and indolent systemic mast cell disease may live a normal life span with symptomatic treatment. Progression to advanced disease is rare, and some improve spontaneously.
- About one-third of patients have systemic mastocytosis with an associated hematologic malignancy. In these cases, the prognosis is related to the ability to manage the hematologic disease. Usually this combination portends a foreshortened life span.
- Elevated serum lactic dehydrogenase and more advanced age tend to be poor prognostic findings.
- Overall 3-year survival is about 50%.

For a more detailed discussion, see Stephen J. Galli, Dean D. Metcalf, Daniel A. Arber, Ann M. Dvorak: Basophils, Mast Cells and Related Disorders, Chap. 63 in *Williams Hematology*, 9th ed.

PART IV

DISORDERS OF MONOCYTES AND MACROPHAGES

CHAPTER 34

Classification and Clinical Manifestations of Monocytes and Macrophages

- Classification is difficult because few diseases result solely in disturbance of monocytes.
- Presence of monocytosis, monocytopenia, or histiocytosis may be an important diagnostic feature and may contribute to functional abnormalities.
- The terms “macrophage” and “histiocyte” are synonymous.
  - — Macrophage is the correct designation when discussing tissue disorders of the mononuclear phagocyte system.
  - — The latter system is the sum of the marrow, blood and tissue pool of monocytes and macrophages. (The term *reticuloendothelial* for this system is obsolete.)
  - — For historical reasons, macrophage disorders are referred to by pathologists as a histiocytic disorder or a histiocytosis (eg, Langerhans cell histiocytosis).

Table 34–1 provides a comprehensive array of disorders of monocytes, macrophages, and (myeloid) dendritic cells. Chapter 35 discusses the various causes for increases and decreases in blood monocytes. Chapters 36 and 37 discuss the principal histiocytic disorders.

TABLE 34–1 DISORDERS OF MONOCYTES AND MACROPHAGES

I. Monocytopenia
- A. Aplastic anemia
- B. Hairy cell leukemia
- C. MonoMAC syndrome
- D. Glucocorticoid therapy

II. Monocytosis
- A. Benign
  - 1. Reactive monocytosis
  - 2. Exercise-induced
- B. Clonal monocytosis
  - 1. Subacute or chronic
    - a. Chronic monocytosis
    - b. Myelodysplastic disorder with monocytosis
  - 2. Progressive
    - a. Acute monocytic leukemia
    - b. Dendritic cell leukemia
    - c. Progenitor cell monocytic leukemia
    - d. Chronic myelomonocytic leukemia
    - e. Juvenile myelomonocytic leukemia

III. Macrophage Deficiency: osteopetrosis (isolated osteoclast deficiency)

IV. Inflammatory Histiocytosis
- A. Primary hemophagocytic lymphohistiocytosis
  - 1. Familial
  - 2. Sporadic
- B. Other inherited syndromes with hemophagocytosis lymphohistiocytosis: Chédiak-Higashi, X-linked lymphoproliferative,

Griscelli
C. Infectious hemophagocytic histiocytosis
D. Tumor-associated hemophagocytic histiocytosis
E. Drug-associated hemophagocytic histiocytosis
F. Disease-associated hemophagocytic histiocytosis
G. Juvenile rheumatoid arthritis (macrophage activation syndrome)
H. Sinus histiocytosis with massive lymphadenopathy

V. Storage Histiocytosis
A. Gaucher disease
B. Niemann-Pick disease
C. Gangliosidosis
D. Sea-blue histiocytosis syndrome

VI. Clonal (Neoplastic) Histiocytosis
A. Langerhans cell histiocytosis
1. Localized
2. Systemic
B. Tumors or sarcomas of histiocytes and dendritic cells
1. Histiocytic sarcoma
2. Langerhans cell sarcoma
3. Interdigitating dendritic cell sarcoma
4. Follicular dendritic cell sarcoma

VII. Monocyte and Macrophage Dysfunction
A. α1-Proteinase inhibitor deficiency
B. Chédiak-Higashi syndrome
C. Chronic granulomatous disease
D. Chronic lymphocytic leukemia
E. Disseminated mucocutaneous candidiasis
F. Glucocorticoid therapy
G. Kawasaki disease
H. Malakoplakia
I. Mycobacteriosis syndrome
J. Leprosy
K. Posttraumatic
L. Septic shock-induced
M. Critically ill subjects
N. Solid tumors
O. Tobacco smoking
P. Marijuana smoking or cocaine inhalation
Q. Whipple disease
R. Human interleukin (IL)-10 effects; Epstein-Barr virus IL-10–like gene product (vIL-10)

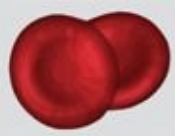
VIII. Atherogenesis

IX. Thrombogenesis

X. Obesity

XI. Aging

Source: *Williams Hematology*, 9th ed, Chap. 69, Table 69–1.



For a more detailed discussion, see Marshall A. Lichtman: Classification and Clinical Manifestations of Monocytes and Macrophages, Chap. 69 in *Williams Hematology*, 9th ed.

CHAPTER 35
Monocytosis and Monocytopenia

- Monocytes in the blood are in transit. They function in the tissues, where they mature into macrophages and participate in:
  — Inflammation, including granulomatous reactions, atheroma formation, and tissue repair
  — Immunologic reactions, including delayed hypersensitivity
  — Reactions to neoplasia and allografts
- The need for macrophages in tissues also can be met by local proliferation of macrophages, not requiring increased transit of blood monocytes.
- Ninety percent of blood monocytes intensely express CD14 (lipopolysaccharide receptor) but not CD16 (Fc receptor) and 10% have weak expression of CD14 and strongly express CD16.
- Older persons have a striking decrease in the proportion of CD14++CD16– to CD14+CD16+ monocytes compared with younger persons.
- Disorders rarely produce abnormalities of monocytes alone in the absence of other blood cell abnormalities.

NORMAL BLOOD MONOCYTE CONCENTRATION

- The monocyte count averages 1.0×10^9/L in neonatal life, gradually decreasing to a mean of 0.4×10^9/L in adult life.
- Monocytosis (in adults) is greater than 0.8×10^9/L.
- Monocytopenia is less than 0.2×10^9/L.

HEMATOLOGIC DISORDERS ASSOCIATED WITH MONOCYTOSIS

- See **Table 35–1**.

TABLE 35–1 DISORDERS ASSOCIATED WITH MONOCYTOSIS

I. Hematologic Disorders
  A. Myeloid neoplasms
    1. Myelodysplastic syndromes
    2. Primary myelofibrosis
    3. Acute monocytic leukemia
    4. Acute myelomonocytic leukemia
    5. Acute monocytic leukemia with histiocytic features
    6. Acute myeloid dendritic cell leukemia
    7. Chronic myelomonocytic leukemia
    8. Juvenile myelomonocytic leukemia
    9. Chronic myelogenous leukemia (m-BCR–positive type)
    10. Polycythemia vera

- 11. Primary myelofibrosis
- B. Chronic neutropenias
- C. Drug-induced neutropenia
- D. Postagranulocytic recovery
- E. Lymphocytic neoplasms
  - 1. Lymphoma
  - 2. Hodgkin lymphoma
  - 3. Myeloma
  - 4. Macroglobulinemia
  - 5. T-cell lymphoma
  - 6. Chronic lymphocytic leukemia
- F. Drug-induced pseudolymphoma
- G. Immune hemolytic anemia
- H. Idiopathic thrombocytopenic purpura
- I. Postsplenectomy state

II. Inflammatory and Immune Disorders
- A. Connective tissue diseases
  - 1. Rheumatoid arthritis
  - 2. Systemic lupus erythematosus
  - 3. Temporal arteritis
  - 4. Myositis
  - 5. Polyarteritis nodosa
  - 6. Sarcoidosis
- B. Infections
  - 1. Mycobacterial infections
  - 2. Subacute bacterial endocarditis
  - 3. Brucellosis
  - 4. Dengue hemorrhagic fever
  - 5. Resolution phase of acute bacterial infections
  - 6. Syphilis
  - 7. Cytomegalovirus infection
  - 8. Varicella-zoster virus
  - 9. Influenza

III. Gastrointestinal Disorders
- A. Alcoholic liver disease
- B. Inflammatory bowel disease
- C. Sprue

IV. Nonhematopoietic Malignancies

V. Exogenous Cytokine Administration

VI. Myocardial Infarction

VII. Cardiac Bypass Surgery

VIII. Miscellaneous Conditions
- A. Tetrachloroethane poisoning
- B. Parturition
- C. Glucocorticoid administration
- D. Depression
- E. Thermal injury
- F. Marathon running
- G. Holoprosencephaly
- H. Kawasaki disease
- I. Wiskott-Aldrich syndrome
- J. Hemodialysis

Source: *Williams Hematology*, 9th ed, Chap. 70, Table 70–1.

Neoplastic or Clonal Monocytic Proliferations

- Oligoblastic myelogenous leukemia (refractory leukemia with excess blasts or myelodysplastic syndrome)
- Acute myelogenous leukemia (myelomonocytic or monocytic types)
- Chronic myelomonocytic leukemia
- Juvenile myelomonocytic leukemia
- Unusual type of *BCR-ABL* (p190)-positive chronic myelogenous leukemia with monocytosis

Reactive (Nonclonal) Monocytic Proliferations

- Neutropenic states: cyclic neutropenia; chronic granulocytopenia of childhood; familial benign neutropenia; infantile genetic agranulocytosis; chronic hypoplastic neutropenia
- Drug-induced agranulocytosis (transient monocytosis, especially in the recovery phase)
- Chlorpromazine toxicity, monocytosis preceding the agranulocytosis
- Lymphoma
- Hodgkin lymphoma
- Postsplenectomy state
- Myeloma

INFLAMMATORY AND IMMUNE DISORDERS ASSOCIATED WITH MONOCYTOSIS

- See **Table 35–1**.

Collagen Vascular Diseases

- Rheumatoid arthritis
- Systemic lupus erythematosus
- Temporal arteritis
- Myositis
- Polyarteritis nodosa

Chronic Infections

- Bacterial infections (eg, subacute bacterial endocarditis, tonsillitis, dental infections, recurrent liver abscesses [probably *not* in typhoid fever or brucellosis])
- Tuberculosis
- Syphilis: neonatal, primary, and secondary
- Viral infections: cytomegalovirus and varicella-zoster virus

Other Inflammatory Disorders

- Sprue
- Ulcerative colitis
- Regional enteritis
- Sarcoidosis (the degree of monocytosis is inversely related to reduction in number of T

lymphocytes)

NONHEMATOPOIETIC MALIGNANCIES

- Found in about 20% of patients who have monocytosis; independent of metastatic disease

MISCELLANEOUS CONDITIONS ASSOCIATED WITH MONOCYTOSIS

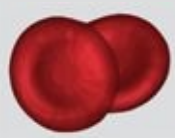
- Alcoholic liver disease
- Tetrachloroethane poisoning
- Langerhans cell histiocytosis
- Parturition
- Severe depression
- See **Table 35–1**

DISORDERS ASSOCIATED WITH MONOCYTOPENIA

- Aplastic anemia
- Hairy cell leukemia:
  — May be a helpful diagnostic clue
  — Contributes to the frequent infections
- Chronic lymphocytic leukemia
- Cyclic neutropenia
- MonoMAC syndrome
  — Caused by *GATA2* mutations
  — Autosomal dominant
  — May be sporadic event
  — Severe monocytopenia (to amonocytosis)
  — B-lymphocytopenia and natural killer cell cytopenia
  — Predisposition to mycobacterial, fungal, human papilloma virus, and Epstein-Barr virus infections
  — Predisposed to develop myelodysplasia or acute myelogenous leukemia
  — Predisposed to develop vulvar carcinoma, metastatic melanoma, cervical carcinoma, Bowen disease of the vulva, and leiomyosarcoma
- Severe thermal injury
- Rheumatoid arthritis
- Systemic lupus erythematosus
- HIV infections
- Postradiation therapy
- Following the administration of:
  — Glucocorticoids
  — α-Interferon
  — Tumor necrosis factor-α

BLOOD DENDRITIC CELLS

- Blood dendritic cells are composed of two phenotypic subtypes: myeloid-derived (HLA-Dr+CD11c+CD123+) and lymphoid-plasmacytoid-derived (HLA-Dr+CD11c–CD123+).
- The total blood dendritic cell count can be measured by flow cytometry.
- Dendritic cells make up approximately 0.6% of blood cells (range: 0.15%–1.30%) and represent 14 × 10^6 cells/L (range: 3 to 30 × 10^6 cells/L). Approximately one-third of these cells are lymphoid-plasmacytoid–derived type and two-thirds are myeloid-derived type.
- Blood dendritic cell counts decrease with aging and increase with surgical stress (and presumably other stressful reactions) in relation to plasma cortisol levels.
- Fluctuations in blood dendritic cells are often independent of changes in blood monocyte counts.



For a more detailed discussion, see Marshall A. Lichtman: Monocytosis and Monocytopenia, Chap. 70 in *Williams Hematology*, 9th ed.

CHAPTER 36
Inflammatory and Malignant Histiocytosis

- The terms *histiocyte* and *macrophage* are synonyms for the mature cell of the monocyte-macrophage system. The latter is the preferred term in discussions of cell biology and immunology, but "histiocytosis" continues to be used by pathologists and in textbooks for diseases of macrophages because of its entrenchment in the medical literature.
- A classification of the histiocytoses most relevant to hematologists is shown in **Table 36–1**. They have been classified based on whether they are monocyte-derived dendritic cell–related, monocyte-macrophage–related, or neoplastic transformations of dendritic cells or macrophages.
- Distinctions among the diseases of macrophages are made based on clinical findings, histopathology, immunophenotyping of surface antigen, cytochemistry, and cytogenetic or genetic features (**Table 36–2**).

TABLE 36–1 CLASSIFICATION OF HISTIOCYTIC DISORDERS

I. Disorders of varying biologic behavior, lacking cytologic atypia
  A. Dendritic-cell-related
    1. Langerhans cell histiocytosis
    2. Juvenile xanthogranuloma
    3. Erdheim-Chester disease
  B. Monocyte-macrophage related
    1. Hemophagocytic lymphohistiocytosis: familial and/or with identified dysfunctional gene mutation
    2. Secondary hemophagocytic syndromes
      a. Infection-associated
      b. Malignancy-associated
      c. Autoimmune-associated
      d. Other
    3. Sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease)
    4. Solitary histiocytoma of macrophage phenotype

II. Malignant disorders
  A. Dendritic cell related
  B. Histiocytic sarcoma
  C. Monocyte-macrophage related
  D. Leukemias: monocytic M5A and M5B, myelomonocytic M4, chronic myelomonocytic leukemia

Source: *Williams Hematology*, 9th ed, Chap. 71, Table 71–2.

TABLE 36–2 DIFFERENTIATING CHARACTERISTICS OF HISTIOCYTES

Histologic Features	LCH	Malignant Histiocytosis	ECD/JXG	HLH	RDD
HLH-DR	++	+	–	+	+
CD1a	++	+/–	–	–	–
CD14	–	+/–	++	++	++

CD68	+/–	+/–	++	++	++
CD163	–	–	+	++	++
CD207 (Langerin)	+++	+/–	–	–	–
Factor XIIIa	–	–	++	–	–
Fascin	–	+/–	++	+/–	+
Birbeck granules	+	+/–	–	–	–
Hemophagocytosis	+/–	–	–	+/–	–
Emperipolesis	–	–	–	–	+

CD, cluster of differentiation; ECD, Erdheim-Chester disease; HLH, hemophagocytic lymphohistiocytosis; JXG, juvenile xanthogranuloma; LCH, Langerhans cell histiocytosis; RDD, Rosai-Dorfman disease.
Source: *Williams Hematology*, 9th ed, Chap. 71, Table 71–1.

CLONAL HISTIOCYTOSES

Langerhans Cell Histiocytosis

Definition and History

- The term *Langerhans cell histiocytosis* includes disorders previously called histiocytosis X (eosinophilic granuloma, Letterer-Siwe disease, Hand-Schüller-Christian disease), self-healing histiocytosis, and Langerhans cell granulomatosis.
- Langerhans cells are macrophages with irregularly shaped nuclei present in epidermis, mucosa, lymph nodes, thymus, and spleen.
- As all macrophages types, they originate in marrow from a multipotential hematopoietic cell.
- Identified by unique racquet-shaped ultrastructural inclusions (Birbeck bodies) and by immunoreactivity to neuroprotein S-100, neuronal specific enolase, and surface antigen CD1a and CD207 (Langerin). Birbeck bodies, CD1a expression, and strong CD207 expression are pathognomonic for Langerhans cells.
- The principal function of Langerhans cells is to process antigen and present it to T cells.

Etiology and Pathogenesis

- Although long thought to be an inflammatory or immunologic disorder, it is now known to be a clonal disease and, therefore, a neoplastic disorder with localized and disseminated forms.
- X chromosome–linked, DNA probes found clonal CD1a+ histiocytes in all lesions tested whether solitary or widespread and whether the lesional cells studied are in one organ or another. In contrast, the T lymphocytes are polyclonal.
- The disease simulates inflammation because the neoplastic Langerhans cells, a dendritic histiocytes, make up a very small fraction of most lesions (≤ 1%).
- The lesion is composed largely of accompanying inflammatory cells (eg, eosinophils, T lymphocytes, macrophages), and is often xanthomatous, fibrotic, or granulomatous, simulating inflammation or infection.
- The presence of $BRAF^{V600E}$ mutations in approximately 35% of a small sample of patients may result in a new therapeutic target in these patients.

Epidemiology

- There are approximately 6 cases per million children younger than age 15 years.
- Disease appears before age 10 years in 75% and before age 30 years in 90% of patients.
- Median age of presentation is 30 months, but disease can present in adults of any age.
- Males frequently have localized disease; 90% of cases with multisystem involvement occur before age 20 years.

Pathogenesis

- Langerhans cell histiocytosis could be a neoplasm based on the compelling evidence in several laboratories for a monoclonal pattern. Studies of adult solitary pulmonary involvement have not found clonal Langerhans cells. Array comparative genomic hybridization did not identify gene mutations in CD207+ cells (presumptive Langerhans cells).
- The Langerhans cells in this disorder behave as an immature dendritic cell and do not stimulate primary T-cell responses effectively. They present antigen poorly and proliferate at a low rate.

Clinical Findings

- Patients may present with single or multiorgan involvement.
- Lesions often involve bone (especially skull and facial bones), skin, lungs, lymph nodes, spleen, thymus, pituitary, and hypothalamus.
- Disease may be localized to a bone or soft tissue site, multifocal in bone only, or multifocal in bone and other sites.
- The disease can occur in any bone. The skull, femurs, ribs, vertebrae (esp. cervical) and humeri are the most frequent sites involved.
- In children, the most frequent site is a lytic lesion of the skull, either painful or not.
- Bony involvement in the face bones has a several-fold risk of central nervous system involvement and diabetes insipidus.
- Diabetes insipidus may appear early or late in the course and is the most frequent sign of central nervous system involvement.
- Diabetes insipidus is the presenting symptom in approximately 4% of patients and 15% to 2% will develop diabetes insipidus during the course of the disease.
- Uncommonly, other central nervous system effects of the disease occur (eg, mass lesions in gray or white matter, neurodegeneration with dysarthria, ataxia, dysmetria). Magnetic resonance imaging may show significant brain changes years before onset of clinical manifestations.
- Cervical lymph nodes most common lymphatic site involved. The thymus and mediastinal nodes may be enlarged.
- Infants may have fever, otitis media, or mastoiditis, with enlargement of liver, spleen, and lymph nodes, or self-limited skin lesions of head and neck.
- Skin lesions can have seborrheic or eczematoid features, and in infants, can be mistaken as prolonged “cradle cap” and in adults as dandruff.
- Skin lesions commonly affect skin flexures in groin, perianal area, the ears, the neck, the armpits, and below the breasts.

- Skin lesions in older children and adults may appear as red papules. Lesions may ulcerate.
- Skin lesions may precede more diffuse disease.
- Oral mucosal lesions may include ulcers on the soft or hard palate, tongue, or lips. There may be gingival hypertrophy.
- Children and adolescents often have pain, tenderness, swelling caused by lytic bone lesion(s); bleeding from gastrointestinal tract; polydipsia; and polyuria as a result of hypothalamic involvement.
- Adult males may have primary pulmonary involvement, causing chronic nonproductive cough, chest pain, dyspnea, wheezing, with a high frequency of associated lung cancer.
- Young women may have localized involvement of the genital tract or it may be part of multicentric involvement. Pregnancy is associated with exacerbation of diabetes insipidus.
- There is a high risk of severe disease in sites including the liver, spleen, lung, and marrow.
- Massive splenomegaly can result in cytopenias.
- Liver enlargement can lead to dysfunction with low albumin, hyperbilirubinemia, and clotting factor deficiencies. Sclerosing cholangitis as a result of bile duct injury is a very serious complication.
- Lung involvement is far more common in adults than children and is associated with cigarette smoking. Chest radiographs show an interstitial infiltrate but computed tomography uncovers cystic and nodular pattern characteristic of Langerhans cell histiocytosis. Later fibrosis can lead to severe pulmonary insufficiency.
- Marrow involvement may be associated with hemophagocytic macrophages as a result of cytokine activation but bone or skin involvement and biopsy results should discriminate between hemophagocytic lymphohistiocytosis and Langerhans cell histiocytosis.
- There is a low risk of severe disease in sites including the skin, bone, lymph nodes, and pituitary gland singly or in any combination.

Laboratory Findings

- Neutrophilia, increased erythrocyte sedimentation rate, increased serum alkaline phosphatase level, and other abnormalities are indicative of liver disease may occur.
- Biopsy of involved tissue demonstrates pathologic Langerhans cells: abundant in proliferative lesions, scarce in fibrotic lesions. Langerhans cells express CD1a and CD207.
- Radiographs, computed tomograms, or positron emission tomograms can identify bone lesions. The latter technique is useful to measure healing of bone after therapy.

Differential Diagnosis

- Depending on site of involvement, this includes chronic granulomatous infections, various infections, lymphoma, collagen vascular disease, pneumoconiosis, and amyloidosis.
- Reactive Langerhans cells may be present in biopsies of Hodgkin lymphoma, malignant lymphoma, and chronic lymphocytic leukemia.

Treatment

- Treatment of **skin disease** is dictated in part by extent of involvement. Topical glucocorticoids usually do not induce a satisfactory response. Oral methotrexate (20 mg/m^2), once weekly, for

6 months or oral thalidomide, 50 to 200 mg, nightly, can be beneficial. Topical nitrogen mustard may benefit those not responding to oral agents, if the disease is not widespread. Psoralen with ultraviolet A light (PUVA) has been used.

- Patients with (1) single skull lesions of the frontal, parietal, or occipital regions or (2) single lesions of any other bone may be treated with curettage or curettage plus injections of methylprednisolone.
- Patients with **skull lesions of mastoid, temporal, or orbital bones** have a risk of developing diabetes insipidus (~35% of untreated patients) and should receive 12 months of treatment with vinblastine, 6 mg/m^2, intravenously, weekly for 7 weeks, and then every 3 weeks, if a good response, coupled with prednisone, 40 mg/m^2, orally, daily, for 4 weeks, then tapered to zero over next 2 weeks. Thereafter, prednisone is given at 40 mg/m^2, orally, daily for 5 days, every 3 weeks with vinblastine injections. If suboptimal response at 6 weeks, vinblastine is continued weekly for an additional 6 weeks before going to every 3 weeks.
- Patients with **femoral or vertebral lesions at risk of collapse** require orthopedic and neurosurgical evaluation. Radiation therapy and a stabilizing orthopedic or neurosurgical procedure, respectively, may be required.
- Patients with **bone lesions or combinations of skin, lymph node, or pituitary gland involvement with or without bone lesions** should receive 12 months of treatment with vinblastine, 6 mg/m^2, intravenously, weekly for 7 weeks, and then every 3 weeks, if a good response, coupled with prednisone, 40 mg/m^2, orally, daily, for 4 weeks, then tapered to zero over next 2 weeks. Thereafter, prednisone is given at 40 mg/m^2, orally, daily for 5 days, every 3 weeks with vinblastine injections. Single-drug treatment is insufficient in this setting and has a high relapse rate.
- Patients with spleen, liver, marrow, or lung (with or without skin, bone, lymph node, or pituitary gland involvement) should receive multidrug therapy as shown in **Table 36–3**.
- Patients with **central nervous system mass lesions** or leptomeningeal involvement have received intravenous cladribine in doses ranging from 5 to 13 mg/m^2 at various frequencies. Also, various other drug combinations have been reported for treatment of neurodegenerative disease associated with Langerhans cell histiocytosis.
- Optimal treatment of children with recurrent or refractory, or progressive disease has not been determined.
- Blood component therapy may be needed for severe cytopenias.
- Splenectomy may be warranted for massive symptomatic splenomegaly and transfusion dependence (hypersplenism).
- Allogeneic hematopoietic stem cell transplantation has been beneficial in some patients with multisystem disease who were refractory to multidrug regimens. Reduced-intensity conditioning can be curative and is associated with less toxicity.
- For isolated bone disease that recurs 6 months or more after the end of treatment, the same treatment program with vinblastine and prednisone can be reused (see above discussion on treatment of bone lesions).
- Recurrent high-risk organ involvement requires one of several multidrug regimens; there is in these cases relatively high treatment-related mortality. Specific approaches have not yet been developed in clinical trials and depend on the organ(s) involved and the duration of the prior

remission.

- For high-risk organ disease, a multidrug program akin to that used for acute myelogenous leukemia or aggressive lymphoma, which includes cytarabine and cladribine, can be used. Allogeneic stem cell transplantation should be incorporated into the latter treatment plan in eligible patients.
- Cladribine and pentostatin have been used in relapsed patients and have frequent salutary effect in those with skin, bone, and lymph node involvement, but less so in patients with liver, marrow, spleen or lung involvement.
- Mutilating surgery of skin, teeth, or jaw bones should not be done as these problems can be managed by systemic chemotherapy, allowing bones to reform.

TABLE 36–3 COMPARISON OF TREATMENT OUTCOMES IN HIGH-RISK LANGERHANS CELL HISTIOCYTOSIS PATIENTS

	LCH-III*	JLSG-96
Number of patients	235	59
Median age at diagnosis	1.1	0.9
Therapy duration (mo)	12	7.5
Initial response (%)	70–72*	76
Reactivations (%)	25–29	45
Survival (mo)	88	97

JLSG-96, Japan Langerhans Cell Histiocytosis Study Group protocol 96; LCH-III, Histiocyte Society Langerhans cell histiocytosis treatment protocol III.

*Arm A (vinblastine/prednisone) vs Arm B (vinblastine/prednisone/methotrexate).

Source: *Williams Hematology*, 9th ed, Chap. 71, Table 71–3.

Course and Prognosis

- Good prognosis is associated with isolated bone lesions.
- Poor prognosis is associated with onset of disease during first 2 years of life, fever not explained by infection, blood cytopenias, and abnormal liver function or pulmonary function tests.
- Refractory multisystem disease is defined as progression on therapy during first 6 weeks or no response to therapy by 12 weeks. Children in this category have approximately a 10% chance of long-term survival.
- Long-term survival (cure) in patients treated with vinblastine and prednisone for low-risk disease is very high, if treatment extends well beyond 6 months.
- Long-term survival in high-risk patients is dependent on a multidrug regimen (see **Table 36–3**), but many can enter long-term remissions. Long-term remissions are in the range of 80% following allogeneic hematopoietic stem cell transplantation.
- About 25% of children with low-risk disease treated successfully have long-term sequelae of therapy. Those with diabetes insipidus are at risk for hypopituitarism (up to 55% with growth hormone deficiency at 10 years follow-up) and should be monitored carefully for growth and development.
- About 75% of patients treated for high-risk disease have long-term problems. Orthopedic problems, hearing loss, neurologic abnormalities, and cognitive defects are among the

sequelae seen. Secondary malignancies after intensive multidrug therapy, pulmonary insufficiency, sclerosing cholangitis, and marrow insufficiency may develop.

Special Characteristics in Adults with Langerhans Cell Histiocytosis

- Manifestations very similar to childhood cases.
- The disease has a median age of presentation of approximately 45 years.
- Isolated adult pulmonary disease has an association with smoking.
- In adults, chest pain may indicate a pneumothorax, which can be the presenting sign of pulmonary disease.
- Long periods of symptoms and signs are often present before a diagnosis is made.
- Presenting symptoms in order of frequency are dyspnea or tachypnea, polydipsia and polyuria, bone pain, weight loss, masses in lymph node areas, fever, gum problems, ataxia, and memory problems.
- Presenting signs in order of frequency are skin rash, scalp nodules, soft-tissue swelling near bone lesions, lymphadenopathy, gingival hypertrophy, hepatosplenomegaly.
- Eighty percent of adult patients presenting with diabetes insipidus had other organ involvement develop over time (eg, bone, skin, lung, lymph nodes).
- Adults have more frequent involvement of the mandible than children (30% vs 7%) and less frequent involvement of the skull than children (21% vs 40%).
- Therapy for adult patients has not been studied in clinical trials.
- Cessation of smoking is very important.
- Adults have frequent severe neuropathic toxicity with vinblastine and do not tolerate prolonged high-dose regimens with glucocorticoids.
- Intravenous cytarabine or cladribine are alternative approaches. The former appears to have a better therapeutic-to-toxicity ratio, but clinical trials have not been done to verify these impressions.
- Oral thalidomide or oral methotrexate have been useful for skin lesions.
- Anecdotes have suggested that intravenous bisphosphonate pamidronate can suppress bone pain in patients with multiple osteolytic lesions.
- Mutilating surgery to remove skin, teeth, or jaw bones is not indicated. These lesions can be dealt with by systemic chemotherapy, allowing bones to reform.
- Lung transplantation has been successful in patients with intractable, disabling pulmonary disease.

Malignant Histiocytosis

Definition

- This rare, rapidly fatal disorder is associated with jaundice, lymphadenopathy, refractory anemia, leukopenia, and hepatosplenomegaly.
- Malignant histiocytosis has been confused with large cell lymphomas. The term *malignant histiocytosis* should be restricted to neoplasms in which the cells have the immunophenotype of histiocytes (see Diagnosis, below).

Clinical Features

- Median age of onset is 35 years.
- Fever, weakness, weight loss, malaise, and sweating are frequent if disease is widespread (see Table 36–4).
- Generalized lymphadenopathy, hepatomegaly, and splenomegaly are frequent.
- Skin, central nervous system, and lung involvement may be associated.
- Localized disease may appear in skin, intestines, or isolated lymph node group.

TABLE 36–4 CLINICAL FEATURES OF MALIGNANT HISTIOCYTIC DISEASES

Histological Type	Signs and Symptoms	Comments
Dendritic or Langerhans cell sarcoma	Fever, weight loss, erythematous nodules, or a skin rash; may involve bone, lymph nodes, lung, liver, and brain.	May evolve from follicular (B-cell) lymphoma.
Extranodal histiocytic sarcoma	Affect soft tissues of extremities (painless masses), gastrointestinal tract (painful masses), nasal cavity, lung, and regional lymph nodes.	Most present as stage I or II tumors.
Interdigitating dendritic cell sarcoma	May be extranodal or lymph nodal. Extranodal cases can involve vertebrae, gastrointestinal tract, marrow, chest wall, and pelvic space.	About 50% present with extranodal disease.
Follicular dendritic cell sarcoma	Nodal and extranodal sites can be involved. Slow growing and painless tumors. Cervical, supraclavicular, and axillary nodes most commonly involved, but mediastinal or mesenteric nodes can be less often.	Metastasis uncommon but can involve lungs.

Laboratory Features

- Anemia and thrombocytopenia present at time of diagnosis in virtually all patients; leukopenia is frequent.
- Marrow examination occasionally shows hemophagocytic macrophages, which are not an important diagnostic feature.
- Increased levels of serum bilirubin and lactic acid dehydrogenase often present. Liver-derived enzymes usually not significantly elevated.
- Elevated serum levels of tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-1α receptor, α_1-antitrypsin, and lysozyme, and angiotensin-converting enzyme are often present.

Diagnosis

- Diagnosis requires lymph node biopsy, marrow aspiration/biopsy, or biopsy of another involved site and unequivocal demonstration of macrophage phenotype and exclusion of B- or T-lymphocyte immunophenotype and T-cell receptor gene rearrangement.
- Neoplastic histiocytes are positive for nonspecific esterase but not for peroxidase reactions, cytochemically.
- Usually can be distinguished by immunophenotype (Table 36–5). In addition, may variably express monocyte-macrophage surface antigens (eg, CD11b, CD11c, CD14, CD15, CD33, CD36).

TABLE 36–5 MORPHOLOGIC AND IMMUNOPHENOTYPIC DISTINCTIONS AMONG MALIGNANT HISTIOCYTIC AND DENDRITIC TUMORS

Phenotypic Feature	Histiocytic Sarcoma (18)	Langerhans Cell Tumor/Sarcoma (26)	Follicular Dendritic Cell Tumor/Sarcoma (13)	Interdigitating Cell Sarcoma (4)

Birbeck granules	Absent	Present	Absent	Absent
Desmosomes	Absent	Absent	Present	Present
Complex cellular junctions	Absent	Absent	Absent	Present
CD1a	0%	100%	0%	0%
S-100 protein	33%	100%	54%	100%
CD21	0%	0%	100%	0%
CD35	0%	0%	100%	0%
CD68	100%	96%	61%	50%

Parentheses indicate number of cases studied. Desmosomes (synonym: macula adherens) are anchoring cell-to-cell junctions that form particularly strong intercellular bonds.
Source: *Williams Hematology,* 7th ed, Chap. 72, Table 72–5.

Differential Diagnosis

- This includes anaplastic large cell lymphomas, which are usually positive for CD30 and may express T-cell or, less often, B-cell antigens; Hodgkin lymphoma; and malignant fibrous histiocytoma, a myofibroblastic tumor that had masqueraded as a histiocytoma on morphologic grounds before marker studies were available.
- This condition is related to other monocyte-macrophage neoplasms (monoblastic leukemia without maturation, if extramedullary involvement is present and marrow is only partially involved). Generally, marrow involvement becomes extensive and leukemia becomes obvious in a short time.
- Langerhans cell sarcoma and follicular dendritic cell tumors are rare malignancies that have been characterized as histiocytic tumors.

Treatment

- There have been no systematic drug trials because of the infrequency of this tumor.
- Multidrug regimens, similar to those used for large-cell lymphomas (eg, drugs such as cyclophosphamide, doxorubicin, vincristine, and prednisone; also bleomycin, teniposide, mechlorethamine hydrochloride, and procarbazine) given at monthly intervals for variable periods have largely been unsuccessful in stage III or IV cases.
- Occasional case reports of responses to oral thalidomide, intravenous alemtuzumab, or a multidrug regimen consisting of mesna, doxorubicin, ifosfamide, and dacarbazine have appeared.
- In some cases, surgical resection of a solitary mass followed by radiation therapy has been successful.
- Remissions are infrequent, but rare long responses (5–7 years) have been observed.
- Allogeneic hematopoietic stem cell transplantation should be considered in eligible patients.

INFLAMMATORY DISORDERS OF HISTIOCYTES

Hemophagocytic Lymphohistiocytosis

Definition

- This progressive and potentially fatal syndrome results from inappropriate activation of lymphocytes and macrophages.
- The pathologic hallmark is macrophages engulfing all types of blood cells in marrow, lymph nodes, spleen, or liver.
- It is also known as autosomal recessive familial lymphohistiocytosis, familial erythrophagocytic lymphohistiocytosis, viral-associated hemophagocytic syndrome, or infection-related hemophagocytic syndrome.
- The designation *primary hemophagocytic lymphohistiocytosis* has been proposed for very young cases with known gene mutations or a family history and secondary or acquired hemophagocytic lymphohistiocytosis for older children, young adults, or those without known gene mutations or for infection-associated cases.
- The overarching term *hemophagocytic lymphohistiocytosis* has been proposed for all because (1) the same mutation may be seen in primary and secondary cases; (2) there is no rapid or definitive gene-testing diagnostic approach; (3) the clinical presentations are the same; and (4) in the acute setting, the distinction is not useful. Both forms should be diagnosed promptly and treated aggressively.

Epidemiology

- In Sweden, annual incidence is 0.12/100,000 children; it occur in 1/50,000 live births.
- In Texas Children's Hospital, there has been 1 case in 3000 inpatient admissions over a 2-year period.
- Males and females are affected equally.
- The condition affects neonates and infants; 90% of cases are children younger than 2 years of age.
- Two-thirds of cases are in siblings; there is frequent parental consanguinity.

Pathogenesis

- Defects in natural killer (NK) and cytotoxic T-cell function lead to inappropriate activation of T cells and macrophages, which secrete proinflammatory cytokines: interferon-γ, TNF-α, IL-6, IL-10, IL-12, soluble IL-2R-α (sCD25).
- Hypercytokinemia ("cytokine storm") results in potentially fatal, severe multiorgan dysfunction.
- Mutation in perforin gene (*PRF1*) leads to decreased perforin elaboration by NK cells and cytotoxic T-lymphocytes. This abnormality decreases apoptosis in target cells resulting in sustained inflammatory response.
- Mutations in other genes in NK and cytotoxic T cells act to decrease apoptosis of target cells, fostering failure to modulate inflammatory reactions.
- Syndromes with high frequency of hemophagocytic lymphohistiocytosis (eg, Chédiak-Higashi syndrome, Hermansky-Pudlak syndrome) have immune deficiencies associated with lysosomal trafficking.
- Epstein-Barr virus infection in setting of immune deficiency state (eg, X-linked lymphoproliferative disorder) can result in hemophagocytic syndrome, a common fatal complication in this circumstance.

Clinical Findings

- In infants, the following findings occur: fever (> 90%), anorexia, vomiting, irritability, and rash (43%).
- Enlarged liver (90%) and spleen (85%) are very common.
- Lymphadenopathy (42%), jaundice, ascites, and edema may occur with progression of disease.
- Meningitis, encephalitic features, seizures, hemiplegia, or coma may ensue.

Laboratory Findings

- Anemia, reticulocytopenia, and thrombocytopenia in most patients at onset; neutropenia less common but with progression of disease, pancytopenia is the rule (**Table 36–6**).
- Marrow eventually contains an increased number of macrophages with prominent phagocytosis of blood cells (hemophagocytic histiocytes). This finding is neither sufficient to make the diagnosis nor is it always present. It is absent in one-third of cases and, thus, is not required for the diagnosis (**Table 36–6**).
- Repeat marrow biopsies as disease progresses may be helpful in uncovering hemophagocytosis, if suspicion of this disease is high.
- Marrow may progress to be hypoplastic with few macrophages and little evident hemophagocytosis.
- The spleen contains swollen macrophages engorged principally with red cells and sometimes leukocytes and platelets.
- Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, lactic dehydrogenase, and triglyceride levels are often elevated.
- Coagulation assays (especially fibrinogen, partial thromboplastin time, and prothrombin time) should be performed and if abnormal further factor assays may be necessary.
- Increased serum concentrations of interferon-γ, TNF, soluble CD8, and IL-6 suggest relationship with cytotoxic T cells and inflammatory cytokines.
- Highly elevated soluble CD25 (soluble IL-2 receptor) greater than 2400 units/mL along with four other criteria shown in **Table 36–6** can be used to make the diagnosis.
- Biopsy of liver or lymph nodes reveals lymphohistiocytic infiltrate with cytologically normal macrophages engorged with phagocytosed erythrocytes (and often leukocytes and platelets). Paracortical lymphoid depletion in lymph nodes is also present.
- Highly elevated serum ferritin (> 500 µg/L) virtually always present, and levels greater than 10,000 µg/L provide 90% sensitivity and 96% specificity for the diagnosis of hemophagocytic lymphohistiocytosis.
- Increasing serial serum ferritin levels are strongly suggestive of lymphohistiocytic hemophagocytic syndrome.
- Highly elevated ferritin (> 500 µg/L) along with four other criteria as shown in **Table 36–6** can be used to make a diagnosis.
- Cerebrospinal fluid examination in patients with nervous system signs may reveal pleocytosis and elevated fluid protein, indicating central nervous system involvement.
- Perforin expression of NK and T cells is decreased.

TABLE 36–6	CLINICAL CRITERIA FOR DIAGNOSIS OF HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

Hemophagocytic lymphohistiocytosis diagnosis is established with at least five of the following:
• Fever
• Splenomegaly
• Cytopenias in at least two cell lines
  Hemoglobin < 90 g/L
  Platelets < 100×10^9/L
  Neutrophils < 1×10^9/L
• Hypertriglyceridemia and/or hypofibrinogenemia:
  Fasting triglycerides > 3 mmol/L (> 265 mg/dL)
  Fibrinogen < 1.5 g/L
• Hemophagocytosis in marrow or spleen or lymph nodes
• Low or absent activity of natural killer cells (specialized laboratory test)
• Ferritin > 500 mcg/L (> 2000 mcg/L may be more specific)
• Soluble cD25 (soluble interleukin-2 receptor) > 2400 U/mL
or
• HLH-associated gene mutations

Source: *Williams Hematology*, 9th ed, Chap. 71, Table 71–4.

Differential Diagnosis

- Moderate infections, sepsis, multiorgan dysfunction, hepatitis, other causes for anemia and thrombocytopenia, and autoimmune diseases such as disseminated lupus erythematosus or rheumatoid arthritis may present with features overlapping the diagnostic findings in hemophagocytic lymphohistiocytosis.
- Consider hemophagocytic lymphohistiocytosis if no clear diagnosis is established and the patient's condition is deteriorating.
- Identification of an underlying immunodeficiency, such as X-linked lymphoproliferative disease, Chédiak-Higashi syndrome, or Griscelli syndrome should increase suspicion of hemophagocytic lymphohistiocytosis.
- Epstein-Barr virus, cytomegalovirus, and other herpes viruses are the most frequent causes of hemophagocytic lymphohistiocytosis. A wide variety of bacterial and fungal infections have been associated with hemophagocytic lymphohistiocytosis.
- The macrophage activation syndrome describes the signs and symptoms of hemophagocytic lymphohistiocytosis in patients with juvenile rheumatoid arthritis or systemic lupus erythematosus.
- The macrophage activation syndrome may include fever, hepatosplenomegaly, mental status changes, cytopenias, and coagulopathy with hypofibrinogenemia. Proliferation of T lymphocytes and macrophages occur and may include poor NK cell function and low perforin expression.

Treatment

- One useful regimen is induction therapy with etoposide and dexamethasone followed by continuous treatment with cyclosporine and pulses of dexamethasone and etoposide.
- Despite neutropenia, etoposide is important, if it can be continued, because it provides singular apoptotic effects on macrophages.
- Cyclosporine levels greater than 200 ng/mL in patients with hypertension or liver or renal disease are dangerous. Such patients may experience seizures and encephalopathy.

- Patients with central nervous system signs or cerebrospinal fluid abnormalities receive intrathecal methotrexate.
- A significant fraction of patients do not respond to initial therapy or have early recurrence. In such patients, while awaiting efforts to use allogeneic hematopoietic stem cell transplantation, escalation of dexamethasone and etoposide therapy or alemtuzumab therapy should be considered because of the high mortality rates associated with initial treatment failure or recurrence. Infliximab, daclizumab, anakinra, and other agents have also been used.
- Treatment is often less intense for patients with HIV-associated hemophagocytic lymphohistiocytosis and patients with juvenile rheumatoid arthritis or systemic lupus erythematosus with the macrophage activation syndrome.
- Anti-TNF-α agents (eg, infliximab or etanercept) can prove useful.
- In patients with the macrophage activation syndrome, therapy should start with cyclosporine and glucocorticoids but not etoposide. The latter should be added only if there is no evidence of improvement after 48 hours of therapy.
- Patients may require support with red cell and platelet transfusions and fresh frozen plasma.
- Prophylactic therapy for *Pneumocystis jiroveci* with sulfamethoxazole and fungi with fluconazole is usually administered.
- Newly diagnosed patients should have human leukocyte antigen typing and a search for a potential related or unrelated donor.
- Allogeneic hematopoietic stem cell transplantation from a histocompatible sibling or unrelated matched donor should be considered in any patient who has resistant disease, relapses, or who has familial hemophagocytic lymphohistiocytosis or a documented relevant gene mutation.
- Reduced–intensity conditioning with allogeneic hematopoietic stem cell transplantation coupled with inclusion of alemtuzumab has shown improved survival and decreased toxicity.

Course

- This syndrome can be rapidly fatal. With current therapy, 3-year survival is approximately 55%.
- Some patients have an initial good response but then relapse as indicated by increase in serum ferritin, coagulation abnormalities, respiratory insufficiency, hypotension, and deteriorating renal function.
- Death is from infection, hemorrhage, or central nervous system abnormalities.

Macrophage Activation Syndrome

- The term is applied to patients with juvenile rheumatoid arthritis or disseminated lupus erythematosus with signs and symptoms simulating hemophagocytic lymphohistiocytosis.
- Macrophage activation is characterized by proliferation of macrophages and T cells, defective NK cell function, and low perforin expression.
- Patients may present with fever, purpura, hepatosplenomegaly, mental status changes, cytopenias, coagulopathy, and hypofibrinogenemia.
- Patients may respond to primary treatment for the underlying autoimmune disease.
- If they do not improve, dexamethasone and etoposide therapy may be tried.

Infection-Induced, Disease-Induced, or Drug-Induced Hemophagocytic Histiocytosis

Etiology

- This condition is usually associated with systemic viral infection; occasionally with bacterial, fungal, or protozoal infections.
- It appears to be an unusual, exaggerated inflammatory reaction to infection.
- It also may occur in association with malignancies.

Clinical Findings

- Fever, severe malaise, myalgias, and lethargy occur.
- Enlargement of liver and spleen is frequent in children but less so in adults.

Laboratory Findings

- Severe anemia (< 90 g/L), leukopenia (< 2.5×10^9/L), thrombocytopenia (< 50×10^9/L), or combination of two cytopenias occur in nearly all cases.
- Interferon-γ, TNF-α, IL-6, and soluble IL-2 receptor plasma levels are markedly increased.
- Macrophages may be present in the blood film.
- Marrow may be hypocellular, with decreased erythropoiesis and granulopoiesis. Increased macrophages frequently contain phagocytosed erythrocytes and erythroblasts and occasionally platelets and neutrophils.

Treatment

- Stop or decrease immunosuppressive therapy in transplant patients.
- If underlying infection, administer appropriate antimicrobial agents.
- Cyclosporine A, antithymocyte globulin, gamma globulin, and etoposide use has been accompanied by improvement in Epstein-Barr virus–associated syndrome.
- In patients with Epstein-Barr virus, rituximab can be useful.

Course

- Patients are severely ill but often recover in weeks with complete disappearance of evidence of histiocytosis in months.
- Fatal outcome is frequent in immunosuppressed patients.

Sinus Histiocytosis with Massive Lymphadenopathy (Rosai-Dorfman Syndrome)

Definition

- This unique entity is characterized by polyclonal (nonmalignant) proliferation of histiocytes with massive lymphadenopathy. It is often self-limited.

Epidemiology

- The syndrome usually occurs in first three decades of life (median age 21 years) but may occur at any age.
- It often occurs in children with an immunologic disorder.

Clinical Findings

- Massive, painless, bilateral cervical lymphadenopathy is characteristic (90% of patients).
- Axillary and inguinal adenopathy develop in 50% of patients.
- Some patients may have fever, night sweats, malaise, and weight loss.
- Extranodal involvement in about 50% of patients. Virtually every tissue may be affected (eg, skin, sinuses, orbit, salivary glands, liver, kidney, bone, and others).
- Bilateral parotid or submandibular gland enlargement may be present.
- Approximately 20% of patients have a maculopapular rash, which can be reddish, bluish, or yellow as a result of xanthomatous involvement.
- Approximately 20% have nasal and paranasal sinus involvement with nasal airway obstruction, epistaxis, nasal septal displacement, or mass lesions invading sinuses.
- Approximately 10% have eyelid or orbital involvement with proptosis.
- Approximately 5% have central nervous system involvement, with intracranial, epidural, or dural masses, or nerve palsies.

Laboratory Findings

- Signs of chronic inflammation (ie, anemia, neutrophilia, elevated erythrocyte sedimentation rate, polyclonal hypergammaglobulinemia) occur.
- Pathologic features in lymph node include:
  — Marked capsular fibrosis and distention of sinuses by phagocytic macrophages are found. Plasma cells are plentiful.
  — Macrophages with ingested intact lymphocytes are virtually diagnostic. Erythrophagocytosis by macrophages is often present.
  — Numerous plasma cells may accompany the macrophages.
  — Macrophages are positive for CD14, CD68, CD163 but are negative for CD1a (**Table 37–2**).
  — Macrophages also express lysozyme, transferrin receptor, and IL-2 receptor.
  — The macrophage proliferation is polyclonal.

Treatment

- The majority of patients will gradually improve without specific treatment.
- Case reports have described improvement in patients with multiorgan involvement necessitating treatment after oral dexamethasone, oral methotrexate, oral 6-mercaptopurine, intravenous cladribine, or intravenous vinorelbine plus methotrexate.
- In patients with bone or central nervous system involvement, intravenous clofarabine has been used.

Course and Prognosis

- Lymph node enlargement usually progresses for weeks to months and then recedes, with no residual evidence of disease after 9 to 18 months.
- Some patients have persistent adenopathy; others may have a fatal outcome.

Erdheim-Chester Disease

Definition and Epidemiology

- This disease results from infiltration of lipid-laden histiocytes that infiltrate bones and viscera, causing a fibroblastic reaction and leading to organ failure.
- The disease affects individuals from childhood to old age and has a mean age of onset of 50 years.
- The etiology is unknown.
- Cells have been determined to be clonal in several cases and polyclonal in several others. Thus, its status as a neoplasm is uncertain.
- The presence of $BRAF^{V600E}$ mutations in approximately 5% of a small sample of patients may result in a therapeutic target in these patients.

Clinical Findings

- Fever, weakness, and weight loss are common presenting complaints.
- Osteosclerosis of long bones is a principal feature.
- Extraosseous involvement occurs in 50% of patients.
- Encasing masses around various organs are characteristic.
- Retroperitoneal and renal involvement causes abdominal pain, dysuria, and hydronephrosis.
- Pulmonary involvement results in dyspnea and pulmonary insufficiency.
- Circumferential sheathing of aorta and coronary arteries may cause cardiac failure.
- Involvement of the pericardium can lead to effusions and effects on myocardial function.
- Diabetes insipidus is a very common presenting disorder.
- Some patients have focal neurologic signs, notably headaches, neuropsychiatric or cognitive difficulties, cranial nerve palsies, and cerebellar dysfunction.
- Bilateral painless exophthalmos may appear.
- Xanthomatous skin lesions may emerge appearing as reddish-brown papules.

Laboratory Findings

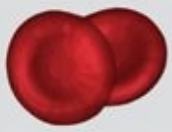
- There are no specific chemical findings.
- Radiographs show bilateral patchy osteosclerosis of the metaphysic and diaphysis of the femur, proximal tibia, and fibula in virtually all patients; lytic bone lesions occur in one-third of patients.
- Computed tomography may show perirenal infiltration extending through perirenal fat giving the appearance of “hair kidneys.”
- Computed tomography may show aortic and aortic branch vessel with circumferential encasement by fibrous tissue.
- Computed tomography may show diffuse interstitial infiltrates.
- The histopathology is distinctive: lipid laden histiocytes with foamy or eosinophilic cytoplasm infiltrating bone and other organs. A fibroblastic reaction is characteristic. The histiocytes express CD68 and factor XIIIa but do not express CD1a and lack Birbeck granules; the latter are specific for Langerhans cell histiocytosis.

Treatment and Course

- No specific regimen has been shown to be effective. Most series have relatively few patients.
- Interferon-α has resulted in significant improvement in some patients in small series and single case reports.
- Glucocorticoids (1 mg/kg per day) have decreased exophthalmos or constitutional symptoms in a small fraction of patients so treated.
- Sixty percent of patients die of their disease and about two-thirds do so within 6 months of diagnosis.
- Mean survival is less than 3 years.
- Cardiac, pulmonary, and renal failure are the principal causes of death.

Juvenile Xanthogranuloma

- This disease of children is usually seen in the first 5 years of life.
- This disorder of dermal dendrocytes forming multiple nodules affect principally the skin of the head, neck, and trunk.
- They are usually skin colored but may be erythematous or yellowish.
- Extracutaneous involvement is rare and systemic symptoms are present only if viscera are involved. Virtually any organ can be affected: nervous system, heart, lung, liver, spleen, intestines, and others. Marrow involvement may cause cytopenias. Pituitary involvement may cause diabetes insipidus.
- Histiocytes are CD14+CD68+ and express factor XIIIa (**Table 36–2**).
- In early disease, intermediate sized histiocytes in sheet-like infiltrations. These cells have small quantities of lipid in the cytoplasm and no Touton-type giant cells (lipid-laden histiocytes with multiple nuclei) with a small amount of centrally located cytoplasm.
- Established disease has abundant foamy histiocytes with Touton giant cells interspersed. Also, there are some lymphocytes and eosinophils.
- If single or few lesions, no therapy is usually needed. Excisional biopsy can be done, if disfiguring.
- In rare cases of systemic disease, a variety of multidrug programs have been used. Inclusion of a vinca alkaloid and a glucocorticoid is recommended.



For a more detailed discussion, see Kenneth L. McClain and Carl E. Allen: Inflammatory and Malignant Histiocytosis, Chap. 71 in *Williams Hematology*, 9th ed.

CHAPTER 37

Gaucher Disease and Related Lysosomal Storage Diseases

GLYCOLIPID STORAGE DISEASES

- These are hereditary disorders in which one or more tissues become engorged with specific lipids because of deficiencies of the lysosomal enzymes required for hydrolysis of one of the glycosidic bonds. The type of lipid and its tissue distribution have a characteristic pattern in each disorder.
- In Gaucher disease (the most common disorder) and Niemann-Pick disease, major clinical manifestations result from macrophage accumulation of glucocerebroside and sphingomyelin, respectively, leading to their massive expansion in tissues.

GAUCHER DISEASE

Etiology and Pathogenesis

- Glucocerebroside accumulates in macrophages because of a deficiency of β-glucocerebrosidase.
- Inheritance is autosomal recessive, with high gene frequency among Ashkenazi Jews.
- More than 100 different mutations have been reported, but the five mutations most common in Ashkenazi Jews account for more than 95% of mutations in that population.
- The most common mutation in the Jewish population is 1226G (N370S). It usually gives rise to mild disease in the homozygous form.

Clinical Features

- Three types of Gaucher disease are recognized based on absence (type 1) or presence of neurological features (types 2 and 3) (see **Table 37–1**).
  - — Type 1 occurs in both children and adults, and is primarily caused by an accumulation of glucocerebroside-laden macrophages in liver, spleen, and marrow. Neurologic manifestations are rare and primarily affect the peripheral nervous system.
  - — Type 2 is exceedingly rare and is characterized by rapid neurologic deterioration and early death.
  - — Type 3, or juvenile Gaucher disease, is a subacute neuropathic disorder with later onset of symptoms and better prognosis than type 2.
- Patients may be asymptomatic, or symptoms may range from minimal to severe:
  - — Chronic fatigue is common.
  - — Hemorrhage occurs after procedures.
  - — Splenic enlargement may cause positional symptoms. Hepatomegaly is usually

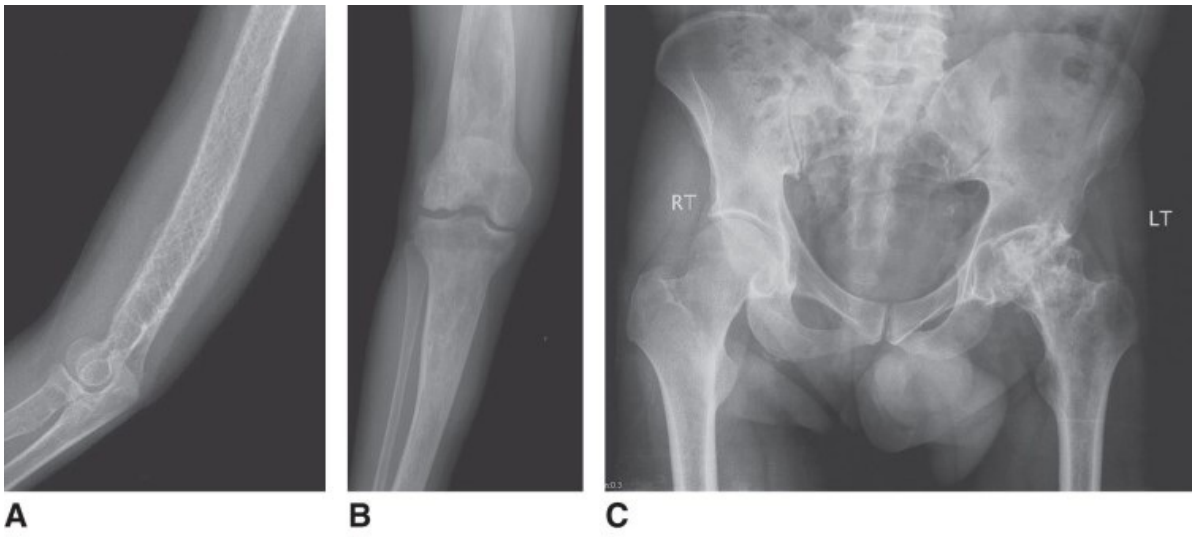
asymptomatic.

— Skeletal lesions are often painful. “Erlenmeyer flask” deformity of the femur is common (Figure 37–1).

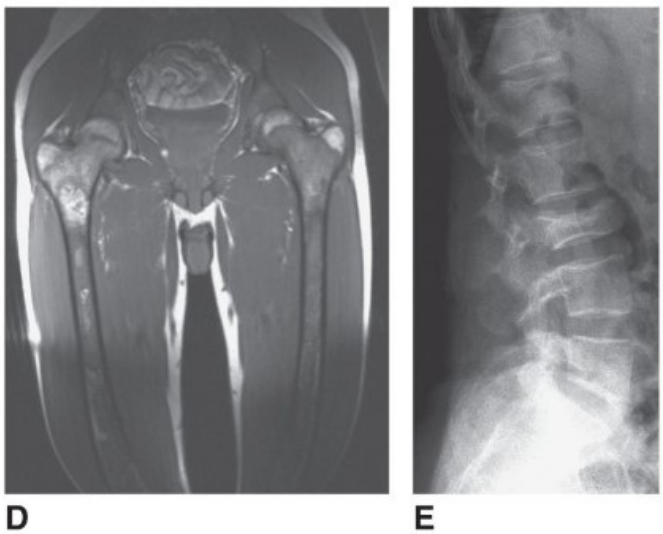
TABLE 37–1 CHARACTERISTICS OF THE THREE TYPES OF GAUCHER DISEASE

	TYPE 1		TYPE 2		TYPE 3		
Subtype	Asymptomatic	Symptomatic	Neonatal	Infantile	3a	3b	3c
Common genotype	N370S/N370S or two mild mutations	N370S/other or two mild mutations	Two null or recombinant mutations	One null and one severe mutations	None	L444P/L444P	D409H/D409H
Ethnic predilection	Ashkenazi Jews	Ashkenazi Jews	None	None	None	Norrbottnians, Asians, Arabs	Palestinian Arabs, Japanese
Common presenting features	None	Hepatosplenomegaly, hypersplenism, bleeding, bone pains	Hydrops fetalis; congenital ichthyosis	SNGP, strabismus, opisthotonus, trismus	SNGP; myoclonic seizures	SNGP; hepatosplenomegaly growth retardation	SNGP; cardiac valves' calcifications
Central nervous system involvement	None	None	Lethal	Severe	SNGP; slowly progressive neurologic deterioration	SNGP; gradual cognitive deterioration	SNGP; brachycephalus
Bone involvement	None	Mild to severe (variable)	None	None	Mild	Moderate to severe; kyphosis (gibbus)	Minimal
Lung involvement	None	None to (rarely) severe	Severe	Severe	Mild to moderate	Moderate to severe	Minimal
Life Expectancy	Normal	Normal/near-normal	Neonatal death	Death before age 3 years	Death during childhood	Death in mid-adulthood	Death in early adulthood

SNGP, supranuclear gaze palsy.
Source: *Williams Hematology*, 9th ed, Chap. 72, Table 72–1.



A B C



D E

FIGURE 37–1 Gaucher-related skeletal involvement. **A.** Humerus with chevron or herringbone pattern. **B.** "Erlenmeyer flask" deformity of the proximal femur. **C.** Plain radiograph of osteonecrosis of the left hip. **D.** Magnetic resonance image of pelvis and thighs that was performed 2 weeks after bone crisis of the right thigh. Bone edema is seen in the upper part of the femur at the level of lesser trochanter. Chronic marrow signal changes are seen in both femurs. **E.** Vertebral collapse. (Used with permission from Dr. Ehud Lebel, Shaare Zedek Medical Center, Jerusalem, Israel.)

Laboratory Features

- Blood counts may be normal or reflect effects of hypersplenism; normocytic, normochromic anemia with modest reticulocytosis is often found. Thrombocytopenia is common, particularly in patients with significant splenomegaly and may be severe.
- Gaucher cells are large cells found in marrow, spleen, and liver in varying numbers. They are characterized by small, eccentrically placed nuclei and cytoplasm with characteristic crinkles or striations. The cytoplasm stains with the periodic acid–Schiff (PAS) technique (**Figure 37–2**).
- Serum acid phosphatase, chitotriosidase, ferritin, and hexosaminidase activities are commonly increased.
- Serum polyclonal gammopathy is common. Monoclonal gammopathies have been found in 1% to 20% of older patients.
- Acquired coagulation factor deficiencies (isolated coagulation factors) have been reported.

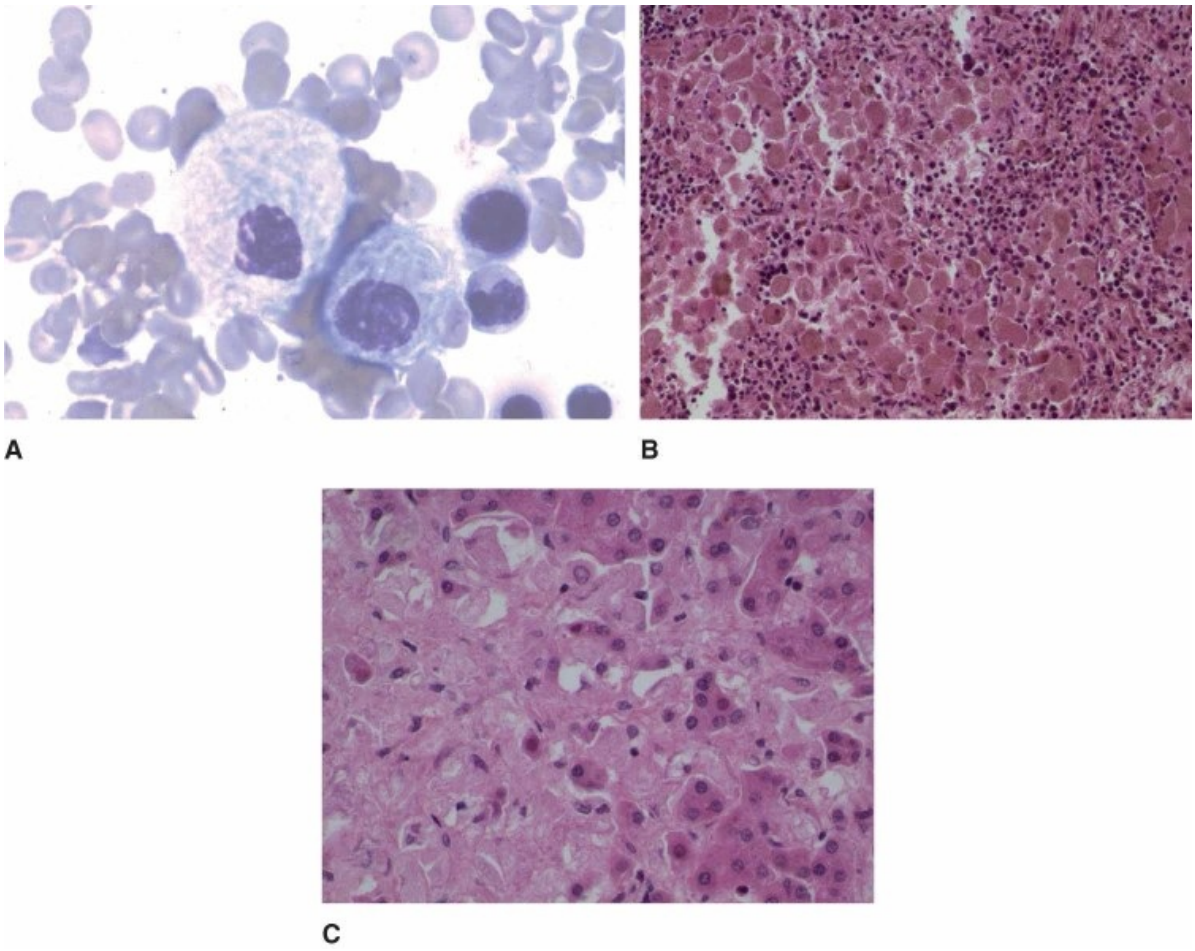


FIGURE 37–2 **A.** "Gaucher cell" in the marrow aspirate of a patient with Gaucher disease. **B.** Histomicrograph of the spleen with marked infiltration of the red pulp by Gaucher cells. **C.** Liver infiltrated by Gaucher cells (the pale pink cells). (Marrow image used with permission from Prof. Chaim Hershko, Shaare Zedek Medical Center, Jerusalem, Israel; spleen and liver images used with permission from Prof. Gail Amir, Hadassah Medical Center, Jerusalem, Israel.)

Diagnosis

- This is established by demonstrating reduced enzymatic activity of β-glucocerebrosidase in leukocytes, cultured fibroblasts, or amniocytes (for prenatal diagnosis).
- Mutational analysis by whole-genome sequencing is recommended.
- Marrow aspiration is indicated only when other hematologic diseases must be considered.
- Definitive diagnosis of heterozygosity must be made by mutational analysis.

Treatment

- Enzyme replacement therapy with recombinant human β-glucosidase (imiglucerase) has been successful. It is very expensive. New enzymes are in clinical trials.
- The enzyme is usually infused with biweekly doses between 15 and 60 U/kg.
- Responses (decrease in liver and spleen size and improved blood counts) usually occur within 6 months. The enzyme does not cross the blood-brain barrier and hence does not affect neuronopathic features.
- In patients who are not suitable for enzyme replacement therapy, oral substrate reduction therapy using oral miglustat (an inhibitor of glucocerebroside synthase) may be considered.
- Future pharmacologic options consist of "chaperone therapy," stabilizing mutant (misfolded) glucocerebrosidase molecules that would otherwise be destroyed prior to their export from the endoplasmic reticulum to the lysosome.
- Splenectomy generally corrects anemia and thrombocytopenia caused by hypersplenism but may cause more rapid deposition of lipid in liver and marrow. Splenectomy is less often performed since the introduction of enzyme replacement therapy.
- Orthopedic procedures, particularly joint replacement, are useful in patients with severe joint damage.
- Hematopoietic stem cell transplantation is curative, but its use is limited by the risk.

Course and Prognosis

- There is often great variability in expression of the disease, even among siblings.
- Severity of the disease changes little after childhood, and progression does not occur or is gradual.
- Some adults with aggressive disease will have slow progression, measured over decades with a gradual fall in platelet count and new bone lesions.
- Pulmonary complications include infiltration of the lungs by Gaucher cells, causing severe interstitial lung disease, usually in patients with severe liver disease and splenectomy. Pulmonary hypertension is rare, can be life-threatening, and does not respond to enzyme replacement therapy.
- There is an increased incidence of malignancies in patients, particularly hematologic malignancies (multiple myeloma) and hepatocellular carcinoma. The latter is seen in cases with severe liver involvement with fibrosis following splenectomy.

NIEMANN-PICK DISEASE

Etiology and Pathogenesis

- This disorder is autosomal recessive.
- Types A and B disease are a consequence of acid sphingomyelinase (ASM) deficiency and are an infantile disease and a disease with later onset, respectively. They are now referred to as ASM deficiency.
- Type C disease is not a result of sphingomyelinase deficiency but rather of mutations in a gene designated *NPC1 or NPC2*, which is involved in cholesterol and glycolipid transport.
- The predominant lipid accumulating in tissues is sphingomyelin in types A and B, and of unesterified cholesterol and several glycolipids in type C.
- Characteristic *foam cells* are found in the lymphoid organs (**Figure 37–3**).

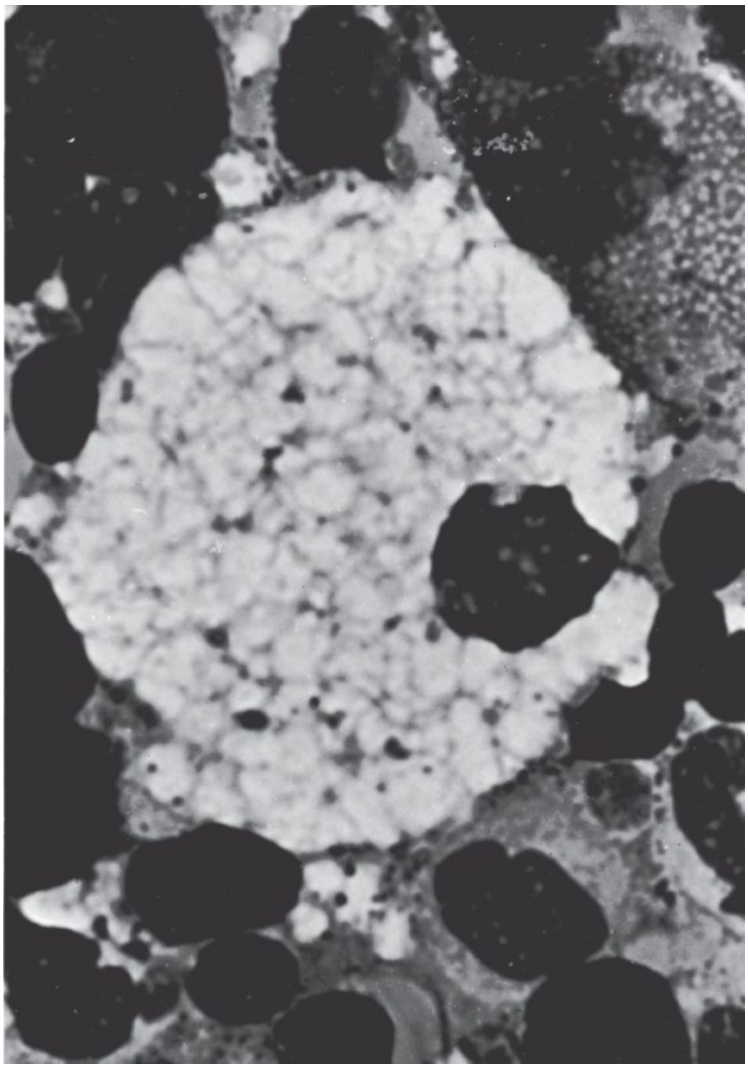


FIGURE 37–3 Typical foam cell from the marrow of a patient with Niemann-Pick disease. (Source: *Williams Hematology*, 9th ed, Chap. 72, Fig. 72–6.)

Clinical Features

- Type A disease presents in infancy with poor growth and neurologic manifestations.
- Type B disease usually presents with hepatosplenomegaly in the first decade of life but in mild cases not until adulthood. Neurologic findings are usually absent but pulmonary involvement is common.
- Type C disease is characterized by neonatal jaundice and dementia, ataxia, and psychiatric

symptoms in later life.

Laboratory Features

- Hemoglobin values may be normal or mild anemia may be present.
- Blood lymphocytes typically contain small, lipid-filled vacuoles.
- Marrow contains foam cells.

Niemann-Pick Types A and B

- Leukocytes or cultured fibroblasts are deficient in sphingomyelinase activity.
- Lipid profiles are always abnormal including high triglycerides and LDL-cholesterol in combination with low HDL cholesterol. The consequences for cardiovascular disease are unknown.

ASM Deficiency and Niemann-Pick Type C

- Large histiocytes containing small lipid droplets (foam cells) or sea-blue histiocytes are demonstrable in many tissues, including marrow.

Diagnosis

- Types A and B disease diagnosed by demonstration that leukocytes or cultured fibroblasts are deficient in sphingomyelinase.
- Heterozygotes for types A and B cannot be reliably detected by measurement of sphingomyelinase activity. Genetic testing needs to be performed.
- Type C disease can be diagnosed by biochemical testing that demonstrates impaired cholesterol esterification and positive filipin staining in cultured fibroblasts. Biochemical testing for carrier status is unreliable. Molecular genetic testing of the *NPC1* and *NPC2* genes detects disease-causing mutations in approximately 95% of individuals with type C disease.

Treatment

- Enzyme replacement therapy is currently being developed for the treatment of Niemann-Pick type B disease.
- Some studies have suggested beneficial effects of miglustat in Niemann-Pick type C disease.

Course and Prognosis

- Patients with type A disease usually die before their third year of life.
- Patients with type B disease may survive into childhood or longer.
- Type C patients usually die in the second decade of life, but some with mild disease have a normal life span.

For a more detailed discussion, see Ari Zimran and Deborah Elstein: Gaucher Disease and Related Lysosomal Storage Diseases, Chap. 72 in *Williams Hematology*, 9th ed.

PART V

PRINCIPLES OF THERAPY FOR NEOPLASTIC HEMATOLOGIC DISORDERS

CHAPTER 38

Pharmacology and Toxicity of Antineoplastic Drugs

BASIC PRINCIPLES OF CANCER CHEMOTHERAPY

- Knowledge of drug actions, clinical toxicities, pharmacokinetics, and drug interactions is essential for the safe and effective administration of cancer chemotherapy.
- Base treatment on evidence from clinical trials.
- Use established regimens and recheck doses.
- Choice of a particular drug treatment program should depend on the disease, histology, and stage of the disease and on an assessment of individual patient tolerance.
- High-dose chemotherapy programs used in autologous and allogeneic hematopoietic cell transplantation result in additional organ toxicities that are not seen at conventional doses.
- Chemotherapy often targets process of DNA replication.
- More recently, drugs have been introduced to target specific cellular processes, including receptor signaling, inhibition of oncoproteins, angiogenesis, and membrane cluster of differentiation antigens.

COMBINATION CHEMOTHERAPY

- Combination chemotherapy uses several drugs simultaneously based on certain empiric principles:
  - — Each drug selected has demonstrable antitumor activity against the neoplasm for which it is used.
  - — Each drug should have a different mechanism of action.
  - — The drugs should not have a common mechanism of resistance.
  - — Drug dose-limiting toxicities should not overlap.
  - — Specific combinations chosen should be based on preclinical and clinical protocol-based evidence of synergistic activity.

CELL KINETICS AND CANCER CHEMOTHERAPY

- Cell cycle–specific agents, such as antimetabolites, kill cells as they traverse the DNA synthetic phase (S phase) of the cell cycle.
  - — Diminished killing of resting cells occurs.
  - — Prolonged exposure to drug is useful for minimizing effects of asynchronous cell division.
  - — High-dose regimens are the most useful.
- Non–cell cycle–dependent agents do not require cells to be exposed during a specific phase of

the cell cycle.
— Total dose of drug is more important than duration of exposure.
— Appropriate dose depends on cell cycle dependence, toxicity to marrow and other tissues, pharmacokinetic behavior, interaction with other drugs, and patient tolerance.

DRUG RESISTANCE

- The basis for drug resistance is spontaneous occurrence of resistant cancer cell mutants and selection of drug-resistant cells under pressure of chemotherapy (clonal selection).
- Mechanisms such as additional mutations in mismatched repair genes and genes that block apoptosis also operate to impair treatment efficacy.
- Use of multiple drugs not sharing resistance mechanisms should be more effective than single agents.
- Multiple agents should be used simultaneously, because the probability of double- or triple-resistant cells is the product of the probabilities of the independent drug-resistant mutations occurring simultaneously in the same cell.

CELL CYCLE–SPECIFIC AGENTS

Methotrexate

- Methotrexate is used for maintenance therapy of acute lymphocytic leukemia, combination chemotherapy of lymphomas, and treatment and prophylaxis of meningeal leukemia.
- This agent inhibits dihydrofolate reductase, which leads to depletion of cellular folate coenzymes and to inhibition of DNA synthesis and cessation of cell replication.
- Acquired resistance is a result of increased levels of dihydrofolate reductase via gene amplification, defective polyglutamylation, and impaired cellular uptake.
- Doses of 5 to 10 mg/m^2 are well absorbed orally, but doses of more than 25 mg/m^2 should be given intravenously.
- The agent is excreted primarily unchanged by the kidney.
- Renal impairment is a contradiction to methotrexate therapy.
- Dose-limiting toxicities are myelosuppression and gastrointestinal effects (mucositis, diarrhea, bleeding).
- Intrathecal methotrexate may result in acute arachnoiditis, delirium, motor deficits, seizures, and coma. Leucovorin cannot prevent or reverse central nervous system (CNS) toxicities.
- Leucovorin intravenously will reverse acute toxicity of methotrexate, except for CNS toxicity.

Cytarabine (Arabinosyl Cytosine, Ara-C)

- This agent is used primarily to treat acute myelogenous leukemia, in combination with an anthracycline antibiotic drug.
- Ara-C triphosphate (Ara-CTP) is formed intracellularly, inhibits DNA polymerase, and causes termination of strand elongation.
- Acquired resistance is a result of a loss of deoxycytidine kinase, the initial activating enzyme of Ara-C, decreased drug uptake, or increased deamination.

- Cytarabine is not active orally and must be given parenterally.
- High cerebrospinal fluid (CSF) concentration achieved (50% of plasma level).
- Cytarabine may be given intrathecally for meningeal leukemia.
- At standard doses (100–150 mg/m^2 per day for 5–10 days), myelosuppression is dose-limiting toxicity.
- High-dose cytarabine therapy, 1 to 3 g/m^2, intravenously, at q12h on days 1, 3, and 5, is especially effective in consolidation therapy of acute myelogenous leukemia.
- At the higher doses (g/m^2), neurologic, hepatic, and gastrointestinal toxicities may occur. Patients older than 50 years of age may develop cerebellar toxicity (ataxia, slurred speech), which can progress to confusion, dementia, and death. Severe conjunctivitis may also occur but may be prevented or reduced by glucocorticoid eye drops.

Gemcitabine

- Although primarily used for solid tumors, gemcitabine, a 2′-2′-difluoro analog of deoxycytidine, has significant activity against Hodgkin lymphoma.
- Its mechanism of action is similar to cytarabine, in that, as a nucleotide, it competes with deoxycytidine triphosphate for incorporation into the elongating DNA strand, where it terminates DNA synthesis.
- Gemcitabine achieves higher nucleotide levels in tumor cells than does Ara-CTP and has a longer intracellular half-life. Standard schedules use 1000 mg/m^2 infused over 30 minutes.

Purine Analogs: 6-Mercaptopurine (6-MP) and 6-Thioguanine (6-TG)

- Both 6-MP and 6-TG are converted to nucleotides by the enzyme hypoxanthine-guanine phosphoribosyl transferase. Cell death correlates with incorporation of the 6-MP or 6-TG nucleotides into DNA.
- Both 6-MP and 6-TG are given orally.
- Equivalent myelosuppression occurs with 6-MP or 6-TG.
- Metabolism of 6-MP is inhibited by allopurinol; 6-TG metabolism is not affected.
- Thiopurine inactivating enzyme activity is decreased in 10% of persons of European descent. Dose adjustment may be necessary.
- Drugs are myelotoxic with peak neutropenia and thrombocytopenia at about 7 days; moderate nausea and vomiting; and mild, usually reversible, hepatotoxicity.

Fludarabine Phosphate

- Fludarabine has outstanding activity in chronic lymphocytic leukemia (CLL). It is strongly immunosuppressive, like the other purine analogs, and is frequently used for this purpose in nonmyeloablative allogeneic hematopoietic cell transplantation.
- It is administered intravenously and eliminated mainly by renal excretion.
- The recommended oral dose is 40 mg/m^2 per day. In CLL, the recommended doses are 25 mg/m^2 per day for 5 days given as 2-hour infusions and repeated every 4 weeks. When administered at these doses, fludarabine causes only moderate myelosuppression.
- At recommended doses, moderate myelosuppression and opportunistic infection are major

toxicities. Peripheral sensory and motor neuropathy may also occur.
- Tumor lysis syndrome may occur with treatment of patients with large tumor burdens. Thus, patients should be well hydrated and their urine alkalinized prior to beginning therapy.

Cladribine (2-Chlorodeoxyadenosine)

- This purine analog is active in hairy cell leukemia, low-grade lymphomas, and CLL. A single course of cladribine, typically 0.09 mg/kg per day for 7 days by continuous intravenous infusion, induces complete response in 80% of patients with hairy cell leukemia and partial responses in the remainder.
- Eliminated primarily (> 50%) by renal excretion. In a patient with renal failure, continuous flow hemodialysis effectively cleared the drug and prevented serious myelosuppression.
- Cladribine retains effectiveness in at least a fraction of hairy cell leukemia patients resistant to deoxycoformycin or fludarabine.
- Myelosuppression, fever, and opportunistic infection are major toxicities.
- Repeated doses may produce thrombocytopenia.

Clofarabine (2-Chloro-2′Fluoro-Arabinosyladenine)

- This analog has halogen substitutions on both the purine ring and arabinose sugar, resulting in a ready uptake and activation to a highly stable intracellular triphosphate, which terminates DNA synthesis, inhibits ribonucleotide reductase, and induces apoptosis.
- As a single agent, the drug is well tolerated by elderly acute myelogenous leukemia (AML) patients in whom it produces remission rates of 30%.
- The usual adult dose of 52 mg/m^2 given as a 2-hour infusion daily for 5 days.
- The primary route of clofarabine clearance is through renal excretion, and dose adjustment according to creatinine clearance is recommended for patients with abnormal renal function.
- Toxicities include myelosuppression; uncommonly, fever, hypotension, and pulmonary edema, suggestive of capillary leak caused by cytokine release; hepatic transaminitis; hypokalemia; and hypophosphatemia.

Nelarabine (6-Methoxy-Arabinosylguanine)

- The only guanine nucleoside analog, nelarabine, has relatively specific activity as a secondary agent for T-cell lymphoblastic lymphoma and acute T-cell leukemias.
- Mode of action is similar to the other purine analogs, in that it becomes incorporated into DNA and terminates DNA synthesis.
- Selective action for T cells may relate to the ability of T cells to activate purine nucleosides and the lack of susceptibility of this drug to purine nucleoside phosphorylase, a degradative reaction.
- Usual doses are an intravenous 2-hour infusion of 1500 mg/m^2 for adults on days 1, 3, and 5, and a lower dose of 650 mg/m^2 per day for 5 days for children.
- The primary toxicities are myelosuppression and abnormal liver function tests, but the drug may cause a spectrum of neurologic abnormalities, including seizures, delirium, somnolence, and the Guillain-Barré syndrome of ascending paralysis.

Pentostatin (2′-Deoxycoformycin)

- A purine analog that inhibits adenosine deaminase, resulting in accumulation of intracellular adenosine and deoxyadenosine nucleotides, which are probably responsible for the cytotoxicity.
- Biweekly doses of 4 mg/m^2 are extremely effective in inducing pathologically confirmed complete responses in hairy cell leukemia.
- Severe depletion of T lymphocytes occurs and opportunistic infections are common.
- The drug is eliminated entirely by the kidney.

Hydroxyurea

- Hydroxyurea inhibits ribonucleotide reductase, which converts ribonucleotide diphosphates to deoxyribonucleotides.
- Hydroxyurea is used to treat polycythemia vera, essential thrombocythemia and primary myelofibrosis, the hyperleukocytic phase of chronic myelogenous leukemia (CML), and to reduce rapidly rising blast counts in the acute phase of CML or in hyperleukocytic AML.
- The drug has also become the standard agent for decreasing the frequency of painful crisis and reducing hospitalization in patients with sickle cell disease and in patients with hemoglobin (Hgb) SC.
- Its antisickling activity results from induction of Hgb F through its activation of a specific promoter for the γ-globin gene. It may also exert antisickling activity and decrease occlusion of small vessels through its generation of the vasodilator nitric oxide or through suppression of neutrophil production and expression of adhesion molecules, such as L-selectin.
- The dose of hydroxyurea is determined empirically; patients are usually started on 500 mg orally per day, and titrated upward to balance disease control and gastrointestinal toxicity in patients with myeloproliferative diseases, and to the limit of mild neutropenia in patients with sickle cell disease.
- Resistance occurs as a result of increases in ribonucleotide reductase activity or from development of a mutant enzyme that binds the drug less avidly.
- The drug is well absorbed when administered orally.
- Renal excretion is major source of elimination.
- Major toxicities are leukopenia and induction of megaloblastic changes in marrow blood cells. Approximately 30% of individuals cannot tolerate hydroxyurea due to gastrointestinal symptoms or skin ulcers.

ANTITUBULINS

Vinca Alkaloids (Vincristine and Vinblastine)

- Vinca alkaloids bind to microtubules and inhibit mitotic spindle formation.
- Resistance occurs by acquisition of multidrug resistance phenotype or development of microtubules with decreased vinca alkaloid binding.
- Vincristine and vinblastine are both administered intravenously. The average single dose of vincristine is 1.4 mg/m^2 and that of vinblastine 8 to 9 mg/m^2. Sequential doses of the drugs are usually given at 1- or 2-week intervals during a cycle of therapy.

- Approximately 70% of vincristine is metabolized in the liver. The site of vinblastine metabolism is unidentified. Liver disease, but not renal disease, requires a reduction in dose. In general, although specific guidelines for dose reduction have not been developed, a 50% decrease in dose is recommended for patients presenting with a bilirubin level of 1.5 to 3 mg/dL and a 75% reduction for levels greater than 3 mg/dL.
- These alkaloids are very useful in Hodgkin or non-Hodgkin lymphomas and acute lymphocytic leukemia (ALL).
- The dose-limiting toxicity of vincristine is neurotoxicity, which may begin with paresthesias of fingers and lower legs and loss of deep-tendon reflexes. Constipation is common. Other severe neurologic effects can occur.
- Severe weakness of extensor muscles of hands and feet may occur with continued use.
- Marrow suppression is not a common side effect of vincristine, but a primary toxicity of vinblastine is leukopenia.
- Both vincristine and vinblastine are potent vesicants on extravasation during administration.
- Neither vincristine nor vinblastine can be given intrathecally.

Taxanes (Paclitaxel and Docetaxel)

- These are antimitotic drugs that bind to microtubules, although the taxanes differ in their mechanism and toxicity profile from the vinca alkaloids.
- These agents have modest activity in lymphoma.
- Both drugs are cleared primarily by hepatic CYP metabolism, although by different isoenzymes (paclitaxel predominantly by CYP 2B6 and docetaxel by CYP 3A4) and are thus cleared more rapidly in patients treated with phenytoin (Dilantin) and other CYP-inducing drugs such as ketoconazole.
- They are formulated in lipid-based solvents that can cause hypersensitivity reactions; therefore, both are administered after pretreatment with antihistamines and glucocorticoids to decrease risk of allergic reaction.
- Principal toxicities are leukopenia, thrombocytopenia, and mucositis.
- Peripheral neuropathy, cardiac arrhythmias, and fluid retention can occur.

TOPOISOMERASE INHIBITORS

Campothecins (Irinotecan and Topotecan)

- These agents target topoisomerase I, preventing resealing of single-strand DNA breaks.
- Irinotecan has activity against some lymphomas.
- Irinotecan, 125 mg/m^2, is administered intravenously once each week for 4 weeks every 42 days.
- Irinotecan should be used with caution in patients with hepatic dysfunction.
- Topotecan, 1.5 mg/m^2 per day for 5 days, may be useful in oligoblastic leukemia, especially subacute myelomonocytic leukemia.
- Topotecan dose should be reduced if renal or hepatic impairment, including patients with Gilbert syndrome.
- Topotecan toxicity is principally myelosuppression and mucositis.

Anthracycline Antibiotics

- Anthracycline antibiotics act by forming a complex with both DNA and the DNA repair enzyme topoisomerase II, resulting in double-stranded DNA breaks.
- Doxorubicin is a mainstay of treatment for Hodgkin disease, and non-Hodgkin lymphoma, in combination with a number of other agents (eg, Adriamycin/bleomycin/vinblastine/dacarbazine [ABVD] and cyclophosphamide/hydroxy-daunorubicin (doxorubicin)/Oncovin/prednisone [CHOP], respectively).
- Daunorubicin and idarubicin are used in combination with cytarabine for AML.
- Mitoxantrone is used for AML.
- The anthracyclines are usually given every 3 to 4 weeks. Schedules that avoid high-peak plasma levels may reduce cardiac toxicity.
- Idarubicin is the only anthracycline that has reasonable oral bioavailability.
- Doxorubicin and daunorubicin are metabolized in the liver. It is wise to begin therapy of patients with elevated serum bilirubin levels at 50% doses of doxorubicin or daunorubicin and adjust according to tolerance.
- Myelosuppression is the major acute toxicity from anthracyclines. Nausea and vomiting may occur.
- Anthracyclines generate intracellular oxygen free radicals, which may cause cardiac toxicity.
- Doxorubicin may produce mucositis.
- All these drugs can produce reaction in previously irradiated tissues.
- All can produce tissue necrosis if extravasated.
- Dose-related chronic cardiac toxicity is a major side effect of doxorubicin and daunorubicin.
- Acute cardiac effects are arrhythmias, conduction disturbances, and pericarditis-myocarditis syndrome.
- Chronic cardiac effects are diminished ejection fraction and clinical congestive heart failure with high mortality.
- Children receiving anthracyclines may show abnormal cardiac development and late congestive heart failure as teenagers.
- Resistance to anthracyclines occurs with increased activity of the MRP protein and the P-glycoprotein transport system, and with altered topoisomerase II activity.

Epipodophyllotoxins

- Two semisynthetic derivatives of podophyllotoxin, VP-16 (etoposide) and VM-26 (teniposide), inhibit topoisomerase II and have significant clinical activity in hematologic malignancies.
- Etoposide is used in combination regimens for Hodgkin lymphoma, large cell lymphomas, leukemias, and various solid tumors, and is a frequent component of high-dose chemotherapy regimens.
- The agents bind to DNA and induce double-stranded breaks.
- Resistance is a result of expression of multidrug resistance phenotype or diminished drug binding.
- They may be given orally or intravenously.
- Clinical activity is schedule dependent. Single conventional doses are ineffective; daily doses for 3 to 5 days are required.

- Hypotension may occur with rapid intravenous administration.
- Major toxicity is leukopenia; thrombocytopenia is less common.
- In high-dose protocols, mucositis is common and hepatic damage may occur.
- Etoposide may induce secondary AML.

AGENTS ACTIVE THROUGHOUT THE CELL CYCLE

Alkylating Drugs

- These are used as single agents or in combination with other drugs to treat hematologic neoplasms.
- All form covalent bonds with electron-rich sites on DNA.
- Myelosuppression and mucositis are the major acute toxicities.
- Pulmonary fibrosis and secondary leukemias are the major delayed toxicities.
- Clinical basis of resistance to alkylating drugs is not fully understood.
- These agents are rapidly eliminated by chemical conjugation to sulfhydryl groups or by oxidative metabolism.
- Cyclophosphamide and ifosfamide produce a toxic metabolite (acrolein) that is excreted in the urine and can cause hemorrhagic cystitis. Acrolein may be detoxified by sodium 2-mercaptoethane sulfonate (mesna) given simultaneously.
- Nitrogen mustard is a potent vesicant.
- Marrow toxicity is cumulative and is a function of the total dose.
- The incidence of secondary leukemias is related to the total dose administered and to the drugs used. Procarbazine is especially potent in inducing secondary leukemia.
- Dose-limiting toxicity of dacarbazine is nausea and vomiting.
- Nitrosoureas produce delayed myelosuppression, with nadir of blood counts 4 to 6 weeks after the dose, and can also cause nephrotoxicity.
- All alkylating agents can produce pulmonary fibrosis. Busulfan and nitrosoureas are the most likely to do so.

High-Dose Alkylating Agent Therapy

- High-dose chemotherapy programs use one or several alkylating agents because of the strong relationship between dose and cytotoxicity of these drugs.
- With autologous or allogeneic hematopoietic stem cell infusions, doses of alkylating agents can be increased 2- to 18-fold until extramedullary toxicities become limiting.

AGENTS OF DIVERSE MECHANISMS

Bleomycin

- Bleomycin is used in combination chemotherapy programs for Hodgkin lymphoma, aggressive lymphomas, or germ cell tumors.
- Antitumor activity is caused by formation of single- and double-stranded DNA breaks.
- Resistance is a result of accelerated drug inactivation, enhanced DNA repair capacity, or decreased drug accumulation.

- The drug is administered intravenously or intramuscularly for systemic effects and may be instilled intrapleurally or intraperitoneally to control malignant effusions.
- It is eliminated largely by renal excretion and may need dose reduction with renal dysfunction.
- It has little effect on normal marrow.
- A major toxicity is pulmonary fibrosis, which is dose related and is usually irreversible.
- Skin changes, also a major toxicity, are dose related, and include erythema, hyperpigmentation, hyperkeratosis, and ulceration.
- Fever and malaise commonly occur.

L-Asparaginase

- L-Asparaginase is used in the treatment of lymphoid neoplasms.
- Neoplastic lymphoid cells require exogenous L-asparagine for growth. L-Asparaginase destroys this essential nutrient.
- L-Asparaginase is given either intravenously or intramuscularly.
- Hypersensitivity reactions vary from urticaria to anaphylaxis. Skin testing with drug may help confirm hypersensitivity. Intramuscular administration may result in fewer hypersensitivity reactions. Patients should be observed carefully after dosing, and epinephrine should be available to reverse acute hypersensitivity reaction.
- Hypoalbuminemia may result from inhibition of hepatic protein synthesis.
- Decreased antithrombin, protein C, and protein S levels may result in arterial or venous thrombosis. Preexisting clotting abnormalities, such as antiphospholipid antibodies or factor V Leiden, may predispose to thromboembolic complications.
- Decreased levels of fibrinogen and factors II, VII, IX, and X may result in bleeding.
- Inhibition of insulin production may result in hyperglycemia.
- High doses of L-asparaginase may cause cerebral dysfunction manifested by confusion, stupor, and coma, and may also cause nonhemorrhagic pancreatitis.
- L-Asparaginase can be used to prevent marrow suppression if given after high-dose methotrexate.

IMMUNOMODULATORY DRUGS

Thalidomide, Lenalidomide, and Pomalidomide

- Thalidomide, and its analogs lenalidomide and pomalidomide, have established value in treating myeloma.
- Lenalidomide is highly active in first-line combination therapy for myeloma and is also approved for treatment of myelodysplasia with the 5q– variant.
- Lenalidomide produces dramatic tumor responses in patients with CLL, including a tumor flare and tumor lysis syndrome, a potentially fatal complication, even in patients with disease refractory to conventional agents. It is equally effective in patients with poor prognostic cytogenetics (chromosomes 11 and 17 deletions).
- The mechanism of actions include a prominent antiangiogenic effect against tumors, immune modulation, inhibition of cytokine (eg, tumor necrosis factor) secretion, and degradation of cereblon.

- The typical oral dose of thalidomide is 50 to 200 mg daily. The major pathways for elimination include spontaneous hydrolysis of the imide esters and further CYP-mediated metabolism by the liver. Less than 1% of the drug is excreted unchanged in the urine.
- Lenalidomide is typically used at oral doses of up to 25 mg/d for 21 of 28 days. It causes much less sedation, constipation, and neurotoxicity than thalidomide, but it does cause prominent myelosuppression in 20% of patients.
- Pomalidomide is given orally in doses up to 4 mg per day. It is eliminated by CYP-mediated metabolism in the liver with minimal renal clearance. Pomalidomide's prominent toxicity is neutropenia in 50% to 60% of patients and thrombocytopenia in 25%. It has little sedating effects. It is highly active in relapsed, refractory myeloma, particularly in combination with dexamethasone and proteasome inhibitors.

DIFFERENTIATING AGENTS

Retinoids

- All-*trans*-retinoic acid (ATRA) may induce a complete response in acute promyelocytic leukemia (APL) by causing maturation and apoptosis of leukemic promyelocytes.
- ATRA is given orally in doses of 25 to 45 mg/m^2 per day in patients with APL.
- ATRA used with an anthracycline antibiotic has increased remission rates and duration of remission in APL.
- Toxicities of ATRA include dry skin, cheilitis, mild but reversible hepatic dysfunction, bone tenderness, hyperostosis on x-ray, and, occasionally, pseudotumor cerebri.
- The "retinoic acid syndrome" may occur, with respiratory failure, pleural and pericardial effusions, and peripheral edema usually associated with a rapid increase in the number of blood neutrophilic cells induced to mature from leukemic promyelocytes. High-dose glucocorticoid therapy may reverse the syndrome if the white blood cell count is rising rapidly. Otherwise, prompt administration of cytotoxic chemotherapy may prevent the syndrome.

Arsenic Trioxide

- Arsenic trioxide induces apoptosis of leukemic cells in APL. Its mechanism of action probably stems from its ability to promote free radical production.
- It can induce a remission and be curative when combined with ATRA in patients with lower-risk APL (diagnostic white blood cell count $< 10 \times 10^9$/L)
- It is useful in refractory APL for reinduction of remission.

EPIGENETIC AGENTS

Demethylating Agents

- Two DNA demethylating agents, azacitidine and decitabine, are approved for treatment of myelodysplastic syndrome and are useful in AML, particularly in older patients and those with comorbidities who cannot tolerate standard induction chemotherapy.

- They are incorporated into DNA with subsequent covalent inactivation of DNA methyltransferase. The resulting inhibition of methylation of cytosine bases in DNA leads to enhanced transcription of otherwise silent genes.
- The usual dose of azacytidine is 75 mg/m^2 subcutaneously or intravenously per day for 7 days, repeated every 28 days, whereas decitabine is used in doses of 20 mg intravenously every day for 5 days every 4 weeks.
- Responses become apparent in patients with myelodysplasia after two to five courses.
- Their principal clinical toxicities are reversible myelosuppression, severe nausea and vomiting, hepatic dysfunction, myalgias, fever, and rash.

Histone Deacetylase Inhibitors

- This family of enzymes removes acetyl groups from amino groups of the lysines found in chromatin, thus promoting the compacting of chromatin and DNA and preventing gene expression.
- Three inhibitors are approved for clinical use: vorinostat, romidepsin, and panobinostat.
- Vorinostat is approved to treat cutaneous T-cell lymphoma, romidepsin is approved to treat cutaneous and peripheral T-cell lymphoma, and panobinostat is approved in combination therapy for treatment of relapsed multiple myeloma.

SMALL MOLECULES WITH SPECIAL MOLECULAR TARGETS

BCR-ABL Tyrosine Kinase Inhibitors

- The first molecularly targeted drug to make a major impact on cancer treatment was imatinib mesylate (Gleevec), an inhibitor of ABL tyrosine kinase activity and notably the mutant ABL characteristic of the BCR-ABL fusion protein in CML.
- The drug has been impressively successful in inducing remission in chronic phase CML, and to lesser degrees in accelerated and blast crisis phases of the disease. However, many patients ultimately develop resistance to imatinib.
- Second-generation agents dasatinib, nilotinib and bosutinib, as well as the third-generation agent ponatinib, are approved for imatinib-refractory or intolerant patients. Targets, unique pharmacokinetics, mechanism of clearance, half-life, dosing, drug interactions, and toxicity are shown in **Table 38–1**.
- The BCR-ABL kinase inhibitors are all well absorbed by the oral route and subject to clearance by hepatic CYP 3A4 metabolism.

TABLE 38–1 **TYROSINE KINASE IN TREATMENT OF CHRONIC MYELOGENOUS LEUKEMIA**

	Targets	Unique Pharmacokinetics	Mechanism of Clearance	Half-Life	Dosing	Drug Interactions	Toxicity
Imatinib	BCR-ABL, c-Kit, platelet-derived growth factor receptor (PDGFR)	98% bioavailability; transport via OCT-1	Hepatic; dose adjustments for severe hepatic and renal impairment	18 hours	Once daily at 400–800 mg	CYP3A4 inducers (eg, dexamethasone, phenytoin, carbamazepine) CYP3A4 inhibitors (eg, aprepitant, clarithromycin, itraconazole)	Dose-related fluid retention, heart failure, hepatotoxicity, nausea and vomiting, diarrhea, abdominal pain, skin reactions, myelosuppression
Dasatinib	BCR-ABL, c-Kit, PDGFR, Src family kinases	pH-dependent absorption	Hepatic	3–5 hours	Once daily at 100 mg or twice daily at 70 mg	CYP3A4 inducers (eg, dexamethasone, phenytoin, carbamazepine) CYP3A4 inhibitors (eg, aprepitant, clarithromycin, itraconazole) Antacids, H2 blockers, proton pump inhibitors	Fluid retention (> 20%) including pleural and pericardial effusions, heart failure, hepatotoxicity, nausea and vomiting, diarrhea, abdominal pain, skin reactions, myelosuppression, QT prolongation (in vitro), hypocalcemia, hypophosphatemia
Nilotinib	BCR-ABL, c-Kit, PDGFR	Increased bioavailability if taken with food	Hepatic	17 hours	Twice daily at 400 mg	CYP3A4 inducers (eg, dexamethasone, phenytoin, carbamazepine) CYP3A4 inhibitors (eg, aprepitant, clarithromycin, itraconazole) Drugs that prolong the QT interval	Fluid retention, heart failure, hepatotoxicity, nausea and vomiting, diarrhea, abdominal pain, skin reactions, myelosuppression, QT prolongation, hypocalcemia, hypophosphatemia, elevated serum lipase and amylase
Bosutinib	BCR-ABL, SRC, LYN, HCK	Absorption may be affected by magnesium intake	Hepatic; dose adjustments for severe hepatic and renal impairment	22 hours	Once daily at 500–600 mg	CYP3A4 inducers (eg, dexamethasone, phenytoin, carbamazepine) CYP3A4 inhibitors (eg, aprepitant, clarithromycin, itraconazole) Antacids, H2 blockers, proton pump inhibitors	Myelosuppression, skin reactions, QT prolongation, Fluid retention, diarrhea, hypophosphatemia, hyper-/hypomagnesemia
Ponatinib	BCR-ABL (including T315I), VEGFR, PDGFR, FGFR, SRC, KIT, RET, TIE-2, FLT-3	pH-dependent absorption	Hepatic	24 hours	Once daily at 30–45 mg	CYP3A4 inducers (eg, dexamethasone, phenytoin, carbamazepine) CYP3A4 inhibitors (eg, aprepitant, clarithromycin, itraconazole) Ponatinib is an inhibitor of ABCG2 and P-glycoprotein	Arterial thrombosis, hepatotoxicity, gastrointestinal perforation, wound healing complications, hemorrhage, myelosuppression, cardiac arrhythmias, pancreatitis

Source: *Williams Hematology*, 9th ed, Chap. 22, Table 22–5.

Janus Kinase Inhibitors (Ruxolitinib)

- The majority of BCR-ABL-negative myeloproliferative neoplasms carry a mutation in the Janus-type tyrosine kinase (*JAK*) gene.
- The substitution mutation $JAK2^{V617F}$ is the most common gain-of-function alteration, and occurs in virtually all cases of polycythemia vera (PV) and approximately 50% of patients with essential thrombocythemia (ET) and primary myelofibrosis (PMF).
- Expression of this mutation results in ligand independent growth or increased sensitivity to the cytokine/growth factor.
- The first specific JAK inhibitor, ruxolitinib, is approved for treatment of PV, ET, and PMF. It inhibits all JAK kinases independent of mutational status.
- Ruxolitinib is orally administered, reduces spleen volume and improve symptom control, but may cause anemia and thrombocytopenia.

Selective Bruton Tyrosine Kinase Inhibitor (Ibrutinib)

- Bruton tyrosine kinase (BTK), a downstream mediator of the B-cell receptor, is critical for B-cell activation, proliferation, and survival.
- Ibrutinib, a selective BTK inhibitor, is highly active in high-risk relapsed CLL and is also approved for patients with mantle cell lymphoma.

- Ibrutinib is orally administered once daily and metabolized by the liver.
- Side effects are typically mild; although 15% of patients develop grades 3 to 4 neutropenia, dose reductions allow drug continuation.

Proteasome Inhibitors (Bortezomib, Carfilzomib)

- Bortezomib inhibits the chymotryptic-like activity of the 20S subunit of the proteasome, thereby altering the balance of intracellular expression of regulators of proliferation and survival in a manner conducive to rapid and irreversible commitment of susceptible cells to their death.
- The drug is highly effective as a single agent in myeloma, due to multiple mechanisms including the induced accumulation of IκB, a proteasomal substrate, and the ensuing IκB inhibition of nuclear factor-κB, which plays an important role in myeloma cell survival.
- Bortezomib has now become a key component of many regimens in which it is combined with other agents, such as prednisone, melphalan, lenalidomide, or thalidomide.
- The standard schedule of bortezomib administration is an intravenous injection at a maximum tolerated dose of 1.3 mg/m^2 administered twice weekly with a 10-day rest period (days 1, 4, 8, 11, 22, 25, 29, and 32).
- The most common side effects are thrombocytopenia and painful sensory neuropathy.

THERAPEUTIC MONOCLONAL ANTIBODIES

- Monoclonal antibodies are an important class of drugs used to treat hematologic malignancies.
- Used alone, they can block access to important growth promoting cell surface molecules; induce apoptosis on binding; promote antibody-dependent cellular cytotoxicity (ADCC); and when coupled to toxic moieties, target cells for enhanced concentrations of the appended molecule.
- See **Table 38–2** for approved drugs, mechanisms, doses and schedules, and major toxicities.

TABLE 38–2 DOSE AND TOXICITY OF FDA-APPROVED MONOCLONAL ANTIBODY-BASED DRUGS

Drug	Mechanism	Dose and Schedule	Major Toxicity
Rituximab	Antibody-dependent cytotoxicity, complement activation, induction of apoptosis	375 mg/m^2 infusion weekly × 4 as single agent, 375 mg/m^2 infusion in combination with chemotherapy	Infusion related; late-onset neutropenia
Ofatumumab	Antibody-dependent cellular cytotoxicity, complement-dependent cytotoxicity	8 weekly followed by 4 monthly infusions during a 24-week period (dose 1 = 300 mg; doses 2 to 12 = 2000 mg)	Infusion related; late-onset neutropenia
Obinutuzumab	Direct cell death and antibody-dependent cellular cytotoxicity	100-mg infusion on cycle 1 day 1, 900 mg on day 2; 1000 mg on days 8 and 15 of cycle 1 and subsequently 1000 mg on day 1 of cycles 2 through 6 in combination with chlorambucil	Infusion reactions, neutropenia
Alemtuzumab	Complement activation,	Escalation 3, 10, 30 mg infusion 3	Infusion-related toxicity with

	antibody-dependent cytotoxicity, possible induction of apoptosis	times per week followed by 30 mg 3 times per week for 4 to 12 weeks	fever, rash, and dyspnea; T-cell depletion with increased infections
Brentuximab vedotin	Antibody drug conjugate comprising an anti-CD 30 monoclonal antibody linked to mono-methylauristatin E	1.8 mg/kg infusion every 3 weeks	Peripheral neuropathy, neutropenia
^{90}Y-ibritumomab tiuxetan	Targeted radiotherapy	0.4 mCi/kg infusion	Hematologic toxicity, myelodysplasia

Source: *Williams Hematology*, 9th ed, Chap. 22, Table 22–6.

Rituximab

- Rituximab was the first monoclonal to receive approval by the US Food and Drug Administration.
- It is a chimeric antibody containing the human immunoglobulin G1 and κ constant regions with murine variable regions that target the B-cell antigen CD20 expressed on the surface of normal B cells and on more than 90% of B-cell neoplasms.
- Rituximab is a component of multiagent chemotherapy for a wide range of lymphomas and other B-cell neoplasms.
- Rarely, rituximab infusion leads to severe mucocutaneous skin reactions (Stevens-Johnson syndrome). Pretreatment with antihistamines, acetaminophen, and glucocorticoids has become a standard measure to modulate infusion reactions.
- Rituximab, as a result of immune suppression, may reactivate hepatitis B infection; patients should be screened for hepatitis B infection prior to initiation of therapy. It may also lead to progressive and fatal multifocal leukoencephalopathy caused by Jacob-Creutzfeldt virus. Hypogammaglobulinemia and delayed neutropenia may appear 1 to 5 months after administration.
- Resistance to rituximab may occur by down regulation of CD20, impaired ADCC, decreased complement activation, limited effects on signaling and induction of apoptosis, or inadequate blood levels.

Ofatumumab

- Ofatumumab is a fully human immunoglobulin G1 kappa monoclonal antibody, which targets a unique CD20 antigen epitope. It has increased affinity for CD20 compared to rituximab.
- Ofatumumab is used to treat CLL refractory to fludarabine and alemtuzumab.

Obinutuzumab

- Obinutuzumab is a glycoengineered type 2 antibody that targets the CD20 antigen.
- Ofatumumab is approved in combination with chlorambucil in the treatment of patients with previously untreated CLL.

Alemtuzumab

- Alemtuzumab (Campath) is a humanized monoclonal antibody targeted against the CD52 antigen present on the surface of normal neutrophils and lymphocytes as well as most B- and

T-cell lymphomas.

- The drug can induce tumor cell death through ADCC and complement-dependent cytotoxicity. The antibody is most useful in treating low-grade lymphomas and CLL, particularly in patients with disease refractory to fludarabine.

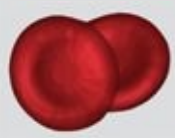
Brentuximab Vedotin

- Brentuximab vedotin is an antibody drug conjugate comprising an anti-CD30 monoclonal antibody conjugated to the potent antimicrotubule agent monomethylauristatin E.
- When brentuximab vedotin binds to CD-30 expressing cells, it is internalized with proteolytic cleavage of the linker, resulting in release of monomethylauristatin E, disrupting the microtubule network and inducing apoptotic cell death.
- It is approved for the treatment of Hodgkin lymphoma after the failure of at least two prior multiagent chemotherapy agents, following failure of an autologous hematopoietic cell transplant, and as maintenance therapy for high-risk patients postautologous transplant.

RADIOIMMUNOCONJUGATES

^{90}Y -Ibritumomab Tiuxetan

- The beta-emitter ^{90}yttrium (^{90}Y) has emerged as an attractive alternative to ^{131}I coupled monoclonal antibodies, based on its higher energy and longer path length as well as its effectiveness in larger tumors. It also has a short half-life and remains tightly conjugated to antibody, even after endocytosis, providing a safer profile for outpatient use.
- ^{90}Y-ibritumomab tiuxetan has responses rates of 65% to 80% in relapsed lymphomas. The major toxicity is hematologic.



For a more detailed discussion, see Benjamin Izar, Dustin Dzube, James M. Cleary, Constantine S. Mitsiades, Paul G. Richardson, Jeffrey A. Barnes, and Bruce A. Chabner: Pharmacology and Toxicity of Antineoplastic Drugs, Chap. 22 in *Williams Hematology*, 9th ed.

CHAPTER 39

Principles of Hematopoietic Cell Transplantation

SOURCES OF HEMATOPOIETIC STEM CELLS

Marrow

- Marrow for hematopoietic cell transplantation (HCT) is typically aspirated by repeated placement of large-bore needles into the posterior iliac crest, generally 50 to 100 aspirations simultaneously on both sides, while under regional or general anesthesia.
- The lowest cell dose to ensure stable long-term engraftment has not been defined with certainty. Typical collections contain more than 2×10^8 total nucleated marrow cells/kg recipient body weight.
- Current guidelines indicate that a volume of up to 20 mL/kg donor body weight is considered safe.
- The risk of serious complications is about 2%.

Peripheral Blood Progenitor Cells

- The most common method to harvest autologous peripheral blood progenitor cells (PBPCs) is by using granulocyte colony-stimulating factor (G-CSF) with or without chemotherapy. In contrast, PBPCs for allogeneic HCT are typically mobilized with G-CSF alone. This procedure is safe and in a review of nearly 7000 healthy unrelated donors, serious side effects were uncommon (< 1%). Splenic rupture is extremely rare.
- The measurement of the absolute number of CD34+ cells/kg recipient body weight collected is a reliable and practical method for determining the adequacy of the PBPC product. A minimum of 2×10^6/kg CD34+ cells is usually recommended, although at this dose, 10% to 20% of autologous collections lead to suboptimal (slow, or more rarely, no) engraftment. Platelet recovery is most sensitive to borderline collection numbers.
- Randomized clinical trials have indicated that engraftment is more rapid with PBPCs than with marrow-derived stem cells.
- Although the number of T cells in peripheral blood stem cell (PBSC) graft is 10-fold greater than in marrow, the incidence of acute graft-versus-host disease (GVHD) does not appear higher, probably because G-CSF influences the proportion of immune tolerizing T_{reg} cells in the apheresis product. However, the risk of chronic GVHD has been found to be about 10% higher in PBSC at most major transplant centers.
- The use of G-CSF to mobilize stem cells in patients with sickle cell anemia is contraindicated, because an acute increase in neutrophil counts can precipitate a catastrophic sickle cell occlusive crisis.

Umbilical Cord Blood

- Umbilical cord blood (UCB) collected from the umbilical vessels in the placenta at the time of delivery is a rich source of hematopoietic stem cells (HSCs).
- Because these cells are immunologically relatively naive, recipients may have satisfactory outcomes, even when crossing major histocompatibility barriers.
- The minimum acceptable cell dose for single-unit UCB transplantation is greater than or equal to 2.5×10^7 total nucleated cells or greater than or equal to 2×10^5 CD34+ cells/kg recipient weight. For adults, this usually requires the use of two suitably matched cord bloods, providing a higher total CD34+ dose.

DEGREE OF MATCHING

Human Leukocyte Antigen (HLA)-Matched Related Donor

- HLA-matched sibling donors were the most common source of HCT products prior to the establishment of sufficiently diverse unrelated stem cell donor banks. The experience with HLA-matched sibling transplants always serves as the major comparison group for alternative HCT donor sources.

HLA-Haploidentical Related Donor

- Because most patients requiring HCT have a haploidentical family donor (on average half of one's siblings and every parent or child), immune depletion of such stem cell products to reduce fatal GVHD has been explored.
- The incorporation of post-transplantation cyclophosphamide as GVHD prophylaxis in T-cell–replete marrow and PBPC HCT has resulted in acceptable rates of acute and chronic GVHD and is well tolerated.
- Numerous studies have now documented the feasibility of this approach. Prospective, randomized clinical trials are underway or planned comparing haploidentical related donor transplant with that of UCB and unrelated donor sources.

HLA-Matched Unrelated Donor (MUD)

- The establishment of diverse banks of individuals willing to donate stem cells should their HLA type be required elsewhere in the world has greatly expanded the likelihood of obtaining a donor for the 60% or greater proportion of individuals for whom a suitable sibling donor cannot be identified. However, the likelihood of obtaining an HLA-matched unrelated donor (MUD) for persons of African or Hispanic descent is substantially lower.
- The only drawbacks to MUD transplants are moderately higher rates of GVHD, secondary to minor histocompatibility antigens less likely to match the transplant recipient if derived from an unrelated rather than a sibling donor; the longer time required to identify a suitable MUD; and the greater possibility (than for a family member) that once identified, a MUD might decline to donate.

HLA Partially Matched Cord Blood Donor

- Because of the immunologic naivete of UCB cells, two or more HLA mismatches are often tolerated for transplantation, resulting in a similar frequency and severity of GVHD as seen using fully matched donors.
- Compared to adult sources of HSCs, UCB transplants engraft more slowly (neutrophils and platelets).

Autologous HSCs

- Autologous HCT is associated with the lowest rate of nonrelapse morbidity and mortality of any transplant strategy.
- The purpose of the infusion of autologous HSCs (referred to as autologous HCT, although not crossing transplantation barriers and is not transplantation in the classic sense) is to permit the administration of very high-dose therapy to induce remission and cure tumors such as leukemia, myeloma, or lymphoma. The autologous HSC infusion restores hematopoiesis and makes mortality from cytotoxic therapy–induced severe and prolonged marrow aplasia unlikely.
- In vivo purging has been widely applied to autologous transplantation. The use of chemotherapy and immunotherapy just prior to mobilization of HSCs reduces tumor cell contamination of the collected product and simultaneously provides a reduction in total tumor burden in the patient prior to his or her high-dose conditioning regimen.

Graft-versus-Tumor Effect

- Compared with autologous transplantation, allogeneic HCT involves more pretransplantation preparation, poses a greater risk of complications to the patient, is associated with a significantly higher nonrelapse morbidity and mortality, and the period of intensive post-transplantation follow-up is considerably longer. However, unlike autologous stem cell products, there is no risk of tumor contamination in allogeneic HSC products.
- Because tumor cells are host derived, and allogeneic immune cells recognize them as foreign, the potential exists for the graft to attack the residual tumor cells (termed a graft-versus-tumor effect) in the patient following conditioning therapy. The evidence that allogeneic HCT outcomes are greatly affected by this graft-versus-tumor effect includes the following: tumor relapse is lower after allogeneic than after syngeneic (identical twin) HCT, tumor relapse is higher in recipients of T-cell–depleted grafts, and donor lymphocyte infusions can induce remission.

CONDITIONING REGIMENS

- A major obstacle to successful allogeneic HCT is the immune competence of the recipient, providing the ability to reject the graft.
- This potential to reject infused donor cells is mediated predominantly through residual host T and natural killer cells.
- In addition to reducing residual tumor burden in transplant recipients, conditioning regimens must reduce or eliminate this immunologic barrier.

Total Body Irradiation

- Total body irradiation (TBI) has been a primary therapeutic modality included in autologous and allogeneic HCT regimens for patients with hematologic malignancies.
- TBI has excellent therapeutic effect against a variety of hematolymphoid malignancies, including leukemia that is resistant to chemotherapy; has sufficient immunosuppressive properties; and is able to treat tumor cell sanctuary sites such as the testicles and the central nervous system.
- Fractionated dose schedules to a total of 1200 to 1320 cGy are typically used.
- TBI is almost always used in conjunction with high-dose chemotherapy (eg, cyclophosphamide) in full-dose conditioning regimens.
- Radiation toxicities include later development of cataracts, treatment-related malignancies, and growth impairment in children.

High-Dose Chemotherapy

- A number of chemotherapy regimens that are both effective for the tumor under treatment and are sufficiently immunologically ablative to allow successful HSC engraftment has been tested in patients undergoing autologous and allogeneic HCT for leukemia and lymphoma.
- For example, BCNU, etoposide and cyclophosphamide alone, busulfan and cyclophosphamide (BuCy), and adjusted dose BuCy in which monitoring of Bu levels reduces its toxicity.
- High-dose melphalan is commonly used for autologous HCT for myeloma.

Reduced-Intensity Conditioning Regimens

- The demonstration that immune-mediated mechanisms are critical in controlling minimal residual disease challenged the concept that relatively toxic full-dose chemoradiation is required for cure following allogeneic HCT.
- Transplantation regimens that use significantly lower doses of chemoradiation, yet that are sufficiently immunosuppressive to allow full donor hematopoietic cell engraftment, have shifted the burden of tumor eradication to graft-versus-tumor effects.
- Reduced-intensity conditioning (RIC) is better tolerated than traditional full-dose regimens and is particularly useful in older patients and in those individuals who have comorbid medical conditions that preclude them from aggressive myeloablative regimens, and for patients without malignancy, in whom it is critical only to allow engraftment of a new immune system.
- One regimen came from detailed studies in a canine model and uses TBI at 2 Gy followed by immunosuppression with mycophenolate mofetil and cyclosporine in an attempt to prevent the recipient T cells from rejecting the graft.
- The addition of fludarabine to low-dose TBI or cyclophosphamide also reduces the risk of graft rejection to less than 5%.
- The risk of GVHD is not reduced by RIC.
- Because host hematopoiesis is not ablated when the patient receives his/her donor HSCs, marrow cells are chimeric soon after transplantation with RIC regimens. However, because host HSCs are at a competitive disadvantage, as they have been irradiated or subjected to toxic drugs during conditioning, over time the donor HSCs progressively populate the entire

marrow.

EVALUATION AND SELECTION OF CANDIDATES FOR TRANSPLANTATION

- Patients considered for transplantation require in-depth counseling by experienced transplantation physicians, nurses, and social workers.
- Information regarding the prior course, including initial diagnostic studies, previous drug and radiation treatments, and responses to these interventions, as well as a psychosocial assessment of the patient and their caregivers, are important.
- **Table 39–1** highlights the issues and topics that should be addressed during the counseling meetings with transplantation candidates and their families or friends.

TABLE 39–1 TOPICS ADDRESSED DURING COUNSELING MEETINGS WITH TRANSPLANT CANDIDATE AND CARE PROVIDER

I. Rationale for why transplantation is a therapeutic option
II. How the transplantation is performed A. Autologous B. Allogeneic—choice for full-dose versus reduced-intensity conditioning
III. Source of cells: marrow versus blood versus other source
IV. Risks of procedure
V. Graft failure and graft rejection
VI. Risk of graft-versus-host disease A. Acute and chronic forms, compatibility of graft B. Likelihood for long-term immune suppression medication
VII. Nonrelapse mortality at 100 days and 1 year
VIII. Risks of relapse
IX. Timing of transplant
X. Projected result
XI. Requirement for dedicated care provider
XII. Other A. Financial implications B. Durable power of attorney C. Banking of sperm, in vitro fertilized eggs D. Duration of stay near the transplantation center E. Return to home and work F. Sexual activity G. Quality-of-life issues H. Habits such as smoking, alcohol, and drug addiction

Source: *Williams Hematology*, 9th ed, Chap. 23, Table 23–2.

DISEASE STATUS AT THE TIME OF TRANSPLANTATION

- Disease status at the time of transplantation is the best predictor of long-term disease-free survival following allogeneic and autologous HCT.

- Attempts at salvaging patients with advanced disease who have failed multiple therapies are rarely successful and if transplantation is to be considered, it is best to consider early in the course of therapy.

AGE AND COMORBID CONDITIONS AT THE TIME OF TRANSPLANTATION

- Among adult and pediatric patients, older age at the time of transplantation is an important determinant that adversely affects nonrelapse mortality following autologous and full-dose allogeneic transplant conditioning.
- Comorbid medical conditions (eg, diabetes mellitus, renal insufficiency) can have a significant impact on transplantation outcomes.
- Routine screening of heart and lung function to detect occult abnormalities is important, especially in older patients.
- Evaluation of liver and kidney function, as well as exposures to potential pathogens such as hepatitis B, hepatitis C, herpes viruses, and human immunodeficiency virus (HIV) should be performed in all patients.

DISEASES TREATED WITH HEMATOPOIETIC CELL TRANSPLANTATION

- In general terms, autologous transplantation is recommended for patients whose malignancy exhibits chemosensitivity to conventional dose therapy and does not extensively involve the marrow.
- In contrast, allogeneic transplantation is generally pursued for hematologic malignancies and disorders that primarily originate in the marrow, such as acute and chronic leukemia, aplastic anemia, and the myelodysplastic and myeloproliferative syndromes.
- A variety of acquired nonmalignant and congenital disorders can be successfully treated with HCT. Most notable is allogeneic HCT for patients with severe aplastic anemia, for which outstanding results have been achieved for those individuals who have an HLA-matched sibling donor; upward of 80% to 90% of these patients have a complete hematologic remission and a long-term disease-free course. HCT for patients with clinically significant hemoglobin disorders, such as thalassemia major, has been successful, especially in patients without significant liver disease. Likewise, allogeneic HCT is considered a treatment option for young patients with severe forms of sickle cell disease.

SELECTED RESULTS OF STEM CELL TRANSPLANTATION

Acute Myelogenous Leukemia

- Hematopoietic HCT has a significant role in the treatment of acute myelogenous leukemia (AML). Many studies have consistently demonstrated that relapse rates are decreased by allogeneic transplantation. In particular, the likelihood of long-term survival is superior in

patients with primary refractory disease, de novo AML with unfavorable cytogenetics at any stage of their disease, or with intermediate or favorable cytogenetics at first relapse or subsequent stages. Patients at first presentation with favorable cytogenetics should be treated with subtype specific chemotherapy. Whether patients with intermediate prognosis cytogenetics should be treated with allogeneic transplantation in first remission is controversial.

Acute Lymphocytic Leukemia (ALL)

- Virtually all adults with acute lymphocytic leukemia (ALL) and standard or high-risk features (including Ph + ALL) should be treated with allogeneic transplantation in first remission.

Myeloma

- Autologous HCT within the first year of initiating treatment has been the standard of care for patients with newly diagnosed myeloma. Although neither chemotherapy nor autologous HCT produces a cure, event-free and overall survival are prolonged following transplantation when compared to treatment with conventional chemotherapy alone. However, with the introduction of lenalidomide and bortezomib, and the ensuing markedly prolonged remissions they induce, the role of HCT is being reexamined.

Non-Hodgkin Lymphoma

- Patients with chemosensitive moderate- and high-grade lymphoma beyond first complete remission have an improved overall survival with high-dose therapy followed by autologous HCT compared to best-of-care salvage chemotherapy. Improvement in survival for patients with B-cell non-Hodgkin lymphoma may further be achieved with inclusion of rituximab as an in vivo purging strategy and perhaps in the post-transplantation setting. Relapse of lymphoma after autologous transplantation is the major reason for treatment failure. Several studies reported that patients who suffer a relapse of lymphoma after autologous transplantation could still be salvaged and experience long-term survival of greater than 45% using RIC and allogeneic transplantation from matched related and unrelated donors.

COMPLICATIONS OF STEM CELL TRANSPLANTATION

- The first 100 days following the cell infusion is typically the time of greatest risk for recipients of autologous and allogeneic HCT.
- The most common complications of HCT are listed in **Table 39–2**.

TABLE 39–2 COMPLICATIONS OF HEMATOPOIETIC CELL TRANSPLANTATION

Vascular access complications
Graft failure
Blood group incompatibilities and hemolytic complications
Acute graft-versus-host disease
Chronic graft-versus-host disease
Infectious complications
Bacterial infections

Fungal infections
Cytomegalovirus infection
Herpes simplex virus infections
Varicella-zoster virus infections
Epstein-Barr virus infections
Adenovirus, respiratory viruses, human herpes virus -6, -7, -8, and other viruses
Gastrointestinal complications
Mucosal ulceration/bleeding
Nutritional support
Hepatic complications
Sinusoidal obstructive syndrome
Hepatitis: infectious versus noninfectious
Lung injury
Interstitial pneumonitis: infectious versus noninfectious
Diffuse alveolar hemorrhage
Engraftment syndrome
Bronchiolitis obliterans
Kidney and bladder complications
Endocrine complications
Drug–drug interactions
Growth and development
Late-onset nonmalignant complications
Osteoporosis/osteopenia, avascular necrosis, dental problems, cataracts, chronic fatigue, psychosocial effects, and rehabilitation
Secondary malignancies
Neurologic complications
Infectious, transplant conditioning and immune suppression medication toxicities

Source: *Williams Hematology*, 9th ed, Chap. 23, Table 23–3.

Graft Failure

- Graft failure is defined as the lack of hematopoietic cell engraftment following autologous or allogeneic HCT.
- Criteria are predominantly operational and graft failure is divided into primary (early) and secondary (late) phases. Primary graft failure is defined as failure to achieve threshold absolute neutrophil, platelet, and hemoglobin values at any point beyond day 28 post-HCT. Secondary graft failure occurs in patients who initially engraft but subsequently lose graft function in at least two cell lines (more often associated with allogeneic HCT).
- The consequences of graft failure are significant and include a high risk of mortality, often as a consequence of infection and hemorrhage related to cytopenias.

Graft Rejection

- Graft rejection is a subset of primary or secondary graft failure. It is caused by the immune-mediated rejection of allogeneic donor cells by residual host effector cells that occurs because of the genetic disparity between the recipient and the donor.
- The determination of graft rejection requires analysis of blood or marrow for chimerism because graft rejection is defined as the inability to detect a meaningful percentage of donor hematopoietic elements.
- Allogeneic HCT following RIC is associated with incomplete eradication of host hematopoietic elements.

Sinusoidal Obstructive Disease (Veno-occlusive Disease)

- Sinusoidal obstructive syndrome (SOS) is a clinical syndrome of tender hepatomegaly, fluid retention, weight gain, and elevated serum bilirubin that follows autologous or allogeneic HCT.
- The incidence of SOS varies significantly with the intensity of the regimen: from less than 10% with RIC to as high as 50% following regimens that use cyclophosphamide combined with TBI of greater than 14 Gy.

Infections

- Two important measures for reducing infections in immunocompromised transplant recipients are an effective handwashing policy and a strategy for preventing transmission of respiratory infections.
- Screening the blood supply has reduced the incidence of transfusion-related infections, especially hepatitis C virus and cytomegalovirus (CMV) in seronegative recipients.
- The duration of neutropenia and severity of oral and gastrointestinal mucosal damage from the conditioning regimen are risk factors for infection before neutrophil recovery has occurred.
- Following neutrophil recovery, the persistent B- and T-cell–mediated immune deficiency increases susceptibility to opportunistic infections.
- Patients who require ongoing immunosuppressive therapy for the control of chronic GVHD are at risk for recurrent bacteremia with encapsulated bacteria and sinopulmonary infections.
- Fungal infections can be serious complications following HCT and are more commonly observed in recipients of allografts as a result of the requirement for post-transplantation immunosuppression medication. Fluconazole prophylaxis decreases the incidence of invasive and superficial *Candida albicans* infections and may decrease the 100-day mortality in allogeneic HCT recipients.
- Infection from the herpesvirus family members can cause significant morbidity and mortality and is a common phenomenon following HCT. Most of the infections are a result of reactivation and the temporal pattern of reactivation follows a relatively predictable course.
- During the first 100 days after transplantation, patients with viremia are at high risk for developing CMV pneumonitis or gastroenteritis. Prophylaxis with acyclovir or valacyclovir reduces the risk of reactivation. First-line therapy for patients with CMV reactivation requiring therapy is intravenous ganciclovir.

Acute GVHD

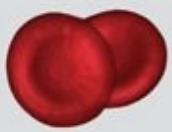
- Acute GVHD remains one of the most serious and challenging complications following allogeneic HCT. The most important risk factor for the development of acute GVHD is the degree of HLA disparity between donor and recipient.
- Acute GVHD typically occurs in the first few weeks to months following transplantation and primarily affects the skin, gastrointestinal tract, and liver. The severity score ranges between grades I and IV and is defined by involvement of each organ system. Grade I consists of a maculopapular skin rash involving less than 50% of body surface area.
- Acute GVHD, grades II to IV, is considered clinically significant because it is moderately severe, and usually consists of multiorgan disease involving the skin, gastrointestinal tract, and

liver. Grade II acute GVHD is not typically associated with a poor outcome. However, grades III and IV acute GVHD are associated with a high risk of mortality and decreased patient survival.

- The mainstay of acute GVHD prevention is prophylaxis with immunosuppressive drugs, and all patients undergoing allogeneic HCT with a T-cell–replete graft require prophylaxis. Primary prophylaxis with cyclosporine-methotrexate or tacrolimus-methotrexate is the commonly used standard to prevent acute GVHD. In the haploidentical HCT setting, post-transplant cyclophosphamide serves as GVHD prophylaxis.
- The most common agent used to treat acute GVHD is a glucocorticoid, usually intravenous methylprednisolone or oral prednisone at a dose of 1 to 2 mg/kg per day with subsequent tapering once disease activity resolves. Glucocorticoids alone are effective in 50% of patients.
- A variety of other approaches have been explored in the treatment of acute GVHD, including the use of other immunosuppressive agents, antibody-based therapies either to T-cells or cytokines, and photopheresis, all administered in combination with prednisone.

Chronic GVHD

- The distinction between acute and chronic GVHD is based on organ involvement and histology rather than time of onset.
- In contrast to acute GVHD, chronic GVHD can affect nearly any organ system. The clinical manifestations are broad and share overlapping features with a variety of autoimmune disorders, such as scleroderma, lichen planus, and dermatomyositis.
- If generalized scleroderma occurs, it may lead to joint contractures and debility.
- Elevations in alkaline phosphatase and serum bilirubin are often the first indication of hepatic involvement with chronic GVHD. Damage to the bile ducts has a similar histopathology to that seen in primary biliary cirrhosis. Liver biopsies are often helpful in establishing a diagnosis.
- The mainstay of treatment for established chronic GVHD continues to be prednisone with or without a calcineurin inhibitor. Because of the chronic nature of this disease, long-term treatment is often required. Alternate-day dosing has been found to help reduce some of the toxicity associated with prolonged glucocorticoid use.



For a more detailed discussion, see Andrew R. Rezvani, Robert Lowsky, and Robert S. Negrin: Principles of Hematopoietic Cell Transplantation, Chap. 21 in *Williams Hematology*, 9th ed.

PART VI

THE CLONAL MYELOID DISORDERS

CHAPTER 40

Classification and Clinical Manifestations of the Clonal Myeloid Disorders

PATHOGENESIS

- These disorders result from a mutation(s) of DNA within a single pluripotential marrow hematopoietic stem cell or very early multipotential progenitor cell. Mutations disturb the function of the gene product(s).
- Overt cytogenetic abnormalities can be found in 50% to 80% of cases of acute myelogenous leukemia (AML) in cytogenetic laboratories (see *Williams Hematology*, 9th ed, Chap. 13, **Figure 13–3**).
  - — Translocations (eg, t(15;17); t(8;21)) and inversions of chromosomes (eg, inv16) can result in the expression of fusion genes that encode fusion proteins that are oncogenic.
  - — Overexpression or underexpression of genes that encode molecules critical to the control of cell growth, or programmed cell death, often within signal transduction pathways or involving transcription factors occur.
  - — Deletions of all or part of a chromosome (eg, -5, 5q-, -7, or -7q) or duplication of all or part of a chromosome may be evident (eg, trisomy 8).
- An early multipotential hematopoietic cell undergoes clonal expansion but retains the ability to differentiate and mature, albeit with varying degrees of pathologic features, into various blood cell lineages (**Figure 40–1**).
- The result is often abnormal blood cell concentrations (either above or below normal), abnormal blood cell structure and function; the abnormalities may range from minimal to severe.
- Resulting disease phenotypes are numerous and varied because of the eight myeloid and three lymphoid differentiation lineages from a multipotential hematopoietic stem cell.
- Neoplasms that result can be grouped, somewhat arbitrarily, by the degree of loss of differentiation and maturation potential and by the rate of disease progression.
- Most patients can be grouped into the classic diagnostic designations listed in **Table 40–1**.

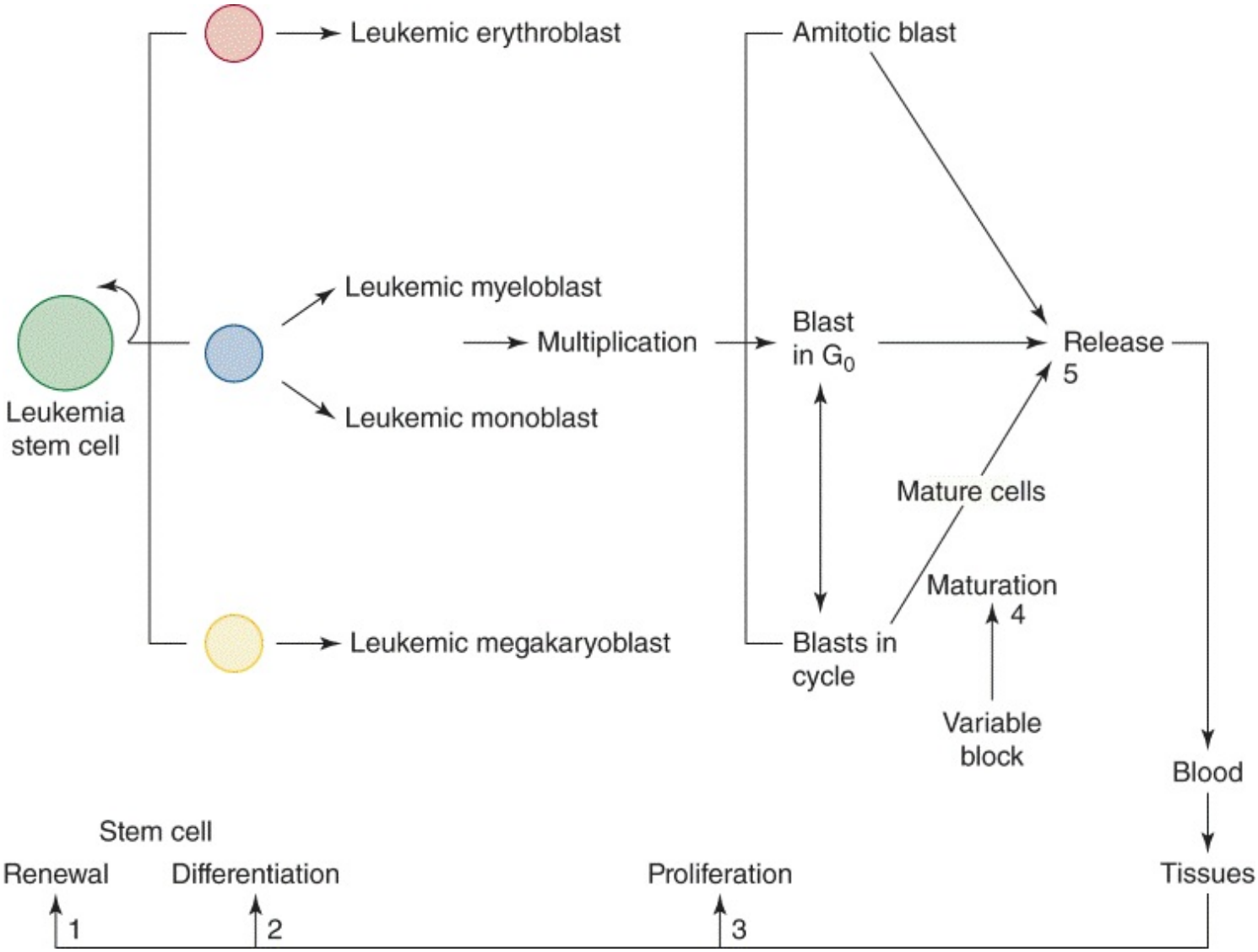


FIGURE 40–1 Leukemic hematopoiesis in acute myelogenous leukemia (AML). The malignant process evolves from a single mutant multipotential cell (possibly a lymphohematopoietic pluripotential cell). This cell, on the basis of a sequence of somatic mutations, becomes a leukemia stem cell with a growth advantage in relationship to normal pluripotential stem cells. Whether all cases of acute myelogenous leukemia originate in the pluripotential stem cell pool is still under study. This cell is capable of multivariate commitment to leukemic erythroid, granulocytic, monocytic, and megakaryocytic progenitors. In most cases, granulocytic and/or monocytic commitment predominates, and myeloblasts and promonocytes or their immediate derivatives are the dominant cell types. Leukemic blast cells accumulate in the marrow. The leukemic blast cells may become amitotic (sterile) and undergo programmed cell death, may stop dividing for prolonged periods (blasts in G_0) but have the potential to reenter the mitotic cycle, or may divide and then undergo varying degrees of maturation. Maturation may lead to mature "leukemic" cells, such as red cells, segmented neutrophils, monocytes, or platelets. A severe block in maturation is characteristic of AML, whereas a high proportion of leukemic primitive multipotential cells mature into terminally differentiated cells of all lineages in patients with chronic myelogenous leukemia. The disturbance in differentiation and maturation in myelogenous leukemia is quantitative, thus many patterns are possible. At least five major steps in hematopoiesis are regulated: (1) stem cell self-renewal, (2) differentiation into hematopoietic cell lineages (eg, red cells, neutrophils, monocytes, megakaryocytes), (3) proliferation (cell multiplication), (4) maturation of progenitor and precursor cells, and (5) release of mature cells into the blood. These control points are defective in AML. Premature or delayed apoptosis of cells may be another key abnormality contributing to premature cell death or cell accumulation. (Source: *Williams Hematology*, 9th ed, Chap. 83, Fig. 83–1.)

TABLE 40–1 NEOPLASTIC (CLONAL) MYELOID DISORDERS

I. Minimal-deviation neoplasms (no increase in blast cells [< 2%] are evident in marrow)
  A. Underproduction of mature cells is prominent
    1. Clonal (refractory sideroblastic or nonsideroblastic) anemia[a]
    2. Clonal bi- or tricytopenia[a]
    3. Paroxysmal nocturnal hemoglobinuria

B. Overproduction of mature cells is prominent
1. Polycythemia vera[b]
2. Essential thrombocythemia[b]

II. Moderate-deviation neoplasms (very small proportions of leukemic blast cells present in marrow)
A. Chronic myelogenous leukemia
1. Philadelphia (Ph) chromosome-positive, BCR rearrangement positive (~90%)
2. Ph chromosome-negative, BCR rearrangement positive (~6%)
3. Ph chromosome-negative, BCR rearrangement negative (~4%)
B. Primary myelofibrosis[b] (chronic megakaryocytic leukemia)
C. Chronic eosinophilic leukemia
1. PDGFR rearrangement-positive
2. FGFR1 rearrangement-positive
D. Chronic neutrophilic leukemia
1. CSF3R-rearrangement-positive
2. CSF3R and SETBP1-rearrangement positive
3. JAK2V617F-rearrangement positive
E. Chronic basophilic leukemia
F. Systemic mastocytosis (chronic mast cell leukemia)
1. KITD816V mutation-positive (~90%)
2. KITV560G mutation-positive (rare)
3. FILIPI-PDGFRα

III. Moderately severe-deviation neoplasms (moderate concentration of leukemic blast cells present in marrow)
A. Oligoblastic myelogenous leukemia (refractory anemia with excess blasts)[a]
B. Chronic myelomonocytic leukemia
1. PDGFR rearrangement positive (rare)
C. Atypical myeloproliferative disease (syn. atypical chronic myelogenous leukemia)
D. Juvenile myelomonocytic leukemia

IV. Severe-deviation neoplasms (leukemic blast or early progenitor cells frequent in the marrow and blood)
A. Phenotypic variants of acute myelogenous leukemia
1. Myeloblastic (granuloblastic)
2. Myelomonocytic (granulomonoblastic)
3. Promyelocytic
4. Erythroid
5. Monocytic
6. Megakaryocytic
7. Eosinophilic[c]
8. Basophilic[d]
9. Mastocytic
10. Histiocytic or Dendritic
B. High-frequency genotypic variants of acute myelogenous leukemia [t(8;21), Inv16 or t(16;16), t(15;17), or (11q23)][e]
C. Myeloid sarcoma
D. Acute biphenotypic (myeloid and lymphoid markers) leukemia[f]
E. Acute leukemia with lymphoid markers evolving from a prior clonal myeloid disease

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[a]The World Health Organization includes these disorders under the rubric of the “Myelodysplastic Syndromes,” the classification of which is discussed in Chap. 44.

[b]The World Health Organization includes these three disorders under the rubric of the “Myeloproliferative Syndromes.”

[c]Acute eosinophilic leukemia is rare. Most cases are subacute or chronic and formerly were included in the category of the hypereosinophilic syndromes.

[d]Rare cases of acute basophilic leukemia are BCR-rearrangement-negative and are variants of acute myelogenous leukemia. Most cases have the BCR rearrangement and evolve from chronic myelogenous leukemia (Chaps. 33, 45, and 46).

[e]The World Health Organization has designated these subtypes as separate entities. They also have phenotypes listed under phenotypic variants. For example, approximately 90% of cases of t(8;21) AML are of the phenotype acute myelogenous leukemia with maturation. Occasional cases are of the phenotypes acute myeloblastic leukemia (no evidence of maturation) or acute myelomonocytic leukemia. Inv(16) is usually an acute myelomonocytic leukemia but can be of other phenotypes, and t(15;17)

invariably manifests itself as an acute promyelocytic leukemia.

[f]Approximately 10% of cases of acute myeloblastic leukemia may be biphenotypic (myeloid and lymphoid CD markers on individual cells) when studied with antimyeloid and antilymphoid monoclonal antibodies (Chap. 45).
Source: *Williams Hematology* 9th ed, Chap. 83, Table 83–1.

MINIMAL TO MODERATE DEVIATION CLONAL MYELOID DISORDERS

- High degree of differentiation and maturation within the clone usually permit a majority of patients to have life spans measured in decades without treatment or with minimally toxic treatment approaches (**Table 40–2**).

TABLE 40–2 **COMPARATIVE SURVIVAL AMONG PERSONS WITH MYELOPROLIFERATIVE NEOPLASMS**

	Percent (%) of Cohort Alive			
Years of Survival	**Expected**	**Essential Thrombocythemia**	**Polycythemia Vera**	**Primary Myelofibrosis**
5	90	90	85	55
10	85	80	70	30
15	75	70	45	30
20	65	50	30	15
25	55	40	20	10

Source: *Williams Hematology*, 9th ed, Chap. 83, Table 83–2.

Ineffective Hematopoiesis (Precursor Apoptosis) is Prominent

- Clonal anemia, bicytopenia, or pancytopenia is the usual manifestation.
- Cytopenias resulting from ineffective hematopoiesis (exaggerated apoptosis) are the most characteristic feature.
- Dysmorphogenesis of blood cells is striking.
- Altered size (macrocytosis and microcytosis) is present.
- Altered shape (poikilocytosis) is present.
- Altered nuclear or organelle structure of blood cells and their precursors (pathologic sideroblasts, acquired Pelger-Hüet neutrophil nuclear malformation, hypogranulation or hypergranulation, abnormal platelet granulation) is apparent.
- Increase in leukemic blast cells in marrow or blood are not evident in these syndromes.
- Blast cells above an upper limit of 2% combined with multilineage abnormalities indicate the presence of oligoblastic myelogenous leukemia (synonym: refractory anemia with excess blasts). The often arbitrary use of a less than 5% blast threshold is not based on classical pathologic diagnostic decisions in which the presence of tumor (leukemic blast) cells is the principal basis for the diagnosis.
- These disorders have a propensity to evolve into polyblastic myelogenous leukemia (eg, AML).

Overproduction of Precursor and Mature Cells Prominent

- Disorders are polycythemia vera and essential thrombocythemia.
- Leukemic blast cells are not present in the marrow or blood.
- Differentiation and maturation in clone is maintained, resulting in functional blood cells.
- Regulation of blood cell concentration is faulty with increased concentrations of some combination of red cells, granulocytes (especially neutrophils), and platelets.
- Survival of cohorts of patients with these diseases is modestly less than expected for age- and gender-matched persons.

MODERATE TO MODERATELY SEVERE DEVIATION CLONAL MYELOID DISORDERS

- Disorders are chronic myelogenous leukemia (CML) and primary myelofibrosis.
- Blast cells are slightly increased in marrow and blood in most patients with these disorders.
- Median life span is usually measured in years but is significantly decreased when compared with age- and gender-matched unaffected cohorts. The introduction into standard therapy of inhibitors of the BCR-ABL oncoprotein (tyrosine kinase inhibitors) has prolonged median survival dramatically in most patients with CML.
- Although virtually all patients with polycythemia vera and approximately 50% percent of patients with essential thrombocythemia and primary myelofibrosis have similar *JAK2* V617 mutational burdens, on average primary myelofibrosis is a more morbid disease with a significantly shorter life expectancy than polycythemia vera or essential thrombocythemia.

MODERATELY SEVERE DEVIATION CLONAL MYELOID DISORDERS

- Oligoblastic myelogenous leukemia (eg, refractory anemia) with excess myeloblasts may occur.
- Chronic myelomonocytic leukemia is such a disease.
  - — This leukemic state has low or moderate concentration of leukemic blast cells in marrow and often blood.
  - — Anemia, often thrombocytopenia, and prominent monocytic maturation of cell is found.
  - — Disorders fall into a group that progress less rapidly than AML and more rapidly than CML.
  - — These subacute syndromes produce more morbidity than do the chronic syndromes and patients have a shorter life expectancy.
- Oligoblastic myelogenous leukemia constitutes about 30% to 50%t of the cases that have been grouped under the designation *myelodysplastic syndromes*.
- Atypical clonal myeloid syndromes (also known as atypical CML) are uncommon syndromes with trilineage abnormalities that do not fall easily into classifiable designations. They are usually seen in patients older than 65 years of age.

SEVERE DEVIATION CLONAL MYELOID DISORDERS

AML and Its Subtypes

- There is an unlimited possibility for phenotypic variation based on the matrix of differentiation of the leukemic multipotential hematopoietic cell and the maturation of leukemic progenitor cells (**Figure 40–2**).
- Subtype alerts the physician to special epiphenomena:
  — Hypofibrinogenic hemorrhage with promyelocytic or monocytic leukemia
  — Tissue and central nervous system infiltration in monocytic leukemia
- Subtype identification requires some or all of the following:
  — Morphology of cells on stained films of blood and marrow
  — Identification of cell antigenic phenotype (CD array) by flow analysis
  — Histochemical characteristics of marrow and blood cells
  — Cytogenetic or molecular diagnostic techniques for recurring genotypes
  — Cytogenetic subclassification, which is more restricted because most cases have any of a variety of infrequently observed abnormalities, making this approach complex
  — Cytogenetic and molecular findings very useful for determining treatment approach, estimating prognosis, and measuring minimal residual disease by polymerase chain analysis, especially in cases in which the cells contain t(8;21), t(15;17), Inv 16, t(16;16), or 11q-

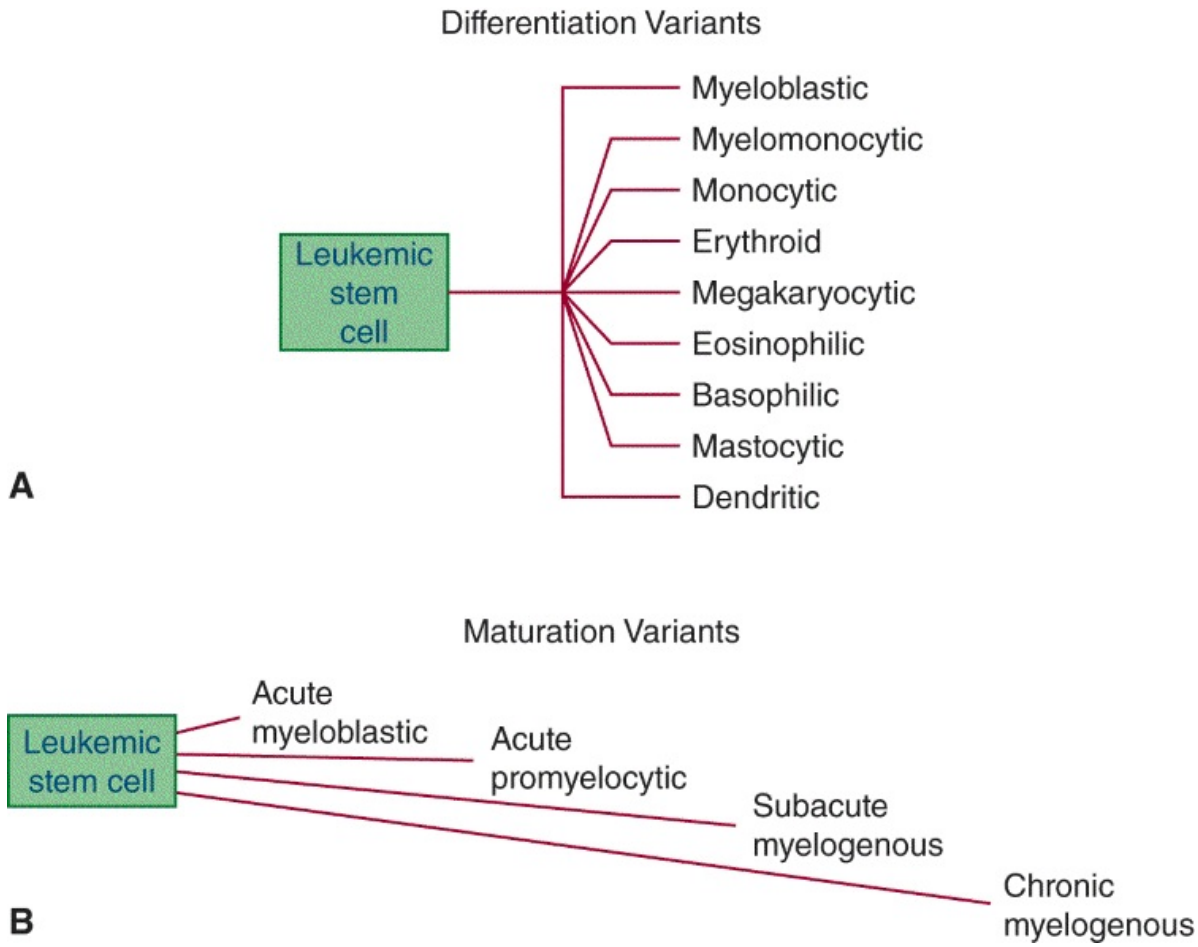


FIGURE 40–2 Phenotypic subtypes of acute myelogenous leukemia. Acute myelogenous leukemia (AML) has variable morphologic expression and a variable degree of maturation of leukemic cells into recognizable precursors of each blood cell type. This phenotypic variation results because the leukemic lesion resides in a multipotential cell capable of all the commitment decisions present normally. **A.** Morphologic variants of AML can be considered differentiation variants in which the cells derived from one of the options of commitment accumulate prominently, although not exclusively (eg, leukemic erythroblasts, leukemic monocytes,

leukemic megakaryocytes). In promyelocytic leukemia and in some cases of acute leukemia in younger individuals, the somatic mutation(s) may arise in a more differentiated progenitor. **B.** Acute myeloblastic leukemia, promyelocytic leukemia, subacute myelogenous leukemia, and chronic myelogenous leukemia can be considered maturation variants in which blocks at different levels of maturation are present in leukemic progenitor cells. Chronic myelogenous leukemia is an example of a leukemia in which differentiation to all lineages occurs and maturation to functional mature cells in each lineage occurs. (Source: *Williams Hematology*, 9th ed, Chap. 83, Fig. 83–3.)

TRANSITIONS AMONG CLONAL MYELOID DISEASES

- Patients with minimal, moderate, and moderately severe clonal myeloid disorders have an increased likelihood of progressing to a more severe syndrome or to florid AML by a process of clonal evolution describing the acquisition of additional cooperating mutations, with a frequency ranging from:
  - — About 1% of patients with paroxysmal nocturnal hemoglobinuria develop AML.
  - — About 10% of patients with clonal anemia (eg, refractory sideroblastic anemia) progress to AML.
  - — About 35% of patients with clonal pancytopenia progress to florid AML.
  - — About 1% to 5% of patients with polycythemia vera not treated with ^{32}P or an alkylating agent (larger proportion of those so treated) develop AML.
  - — About 15% of patients with polycythemia vera evolve to a syndrome indistinguishable from primary myelofibrosis.
  - — About 5% of patients with essential thrombocythemia progress to AML.
  - — About 15% of patients with primary myelofibrosis evolve to AML.
- Most patients with CML progress to acute leukemia as a feature of its natural history. (Current therapy with tyrosine kinase inhibitors has markedly delayed or prevented the onset of acute leukemia in most patients.)
- Patients with CML may enter a phase that behaves like oligoblastic leukemia (accelerated phase) before progression to florid acute leukemia (now much less frequent because of tyrosine kinase therapy).

MULTIPOTENTIAL HEMATOPOIETIC CELL AS SITE OF THE LESION

- Proto-oncogene mutations develop in a multipotential hematopoietic or pluripotential lymphohematopoietic cell and result in most of the clonal myeloid diseases, especially in older patients.
- In CML, the mutation is in a pluripotential lymphohematopoietic cell.
- In other syndromes, the evidence for involvement of B- and T-lymphocyte lineages or multiple myeloid lineages is inconsistent.
- In AML, there is evidence for three levels of onset: pluripotential, multipotential, and bipotential progenitor cells.

PROGENITOR CELL LEUKEMIA

- Leukemic transformation in some (young) patients can occur in progenitor cells (eg, colony-

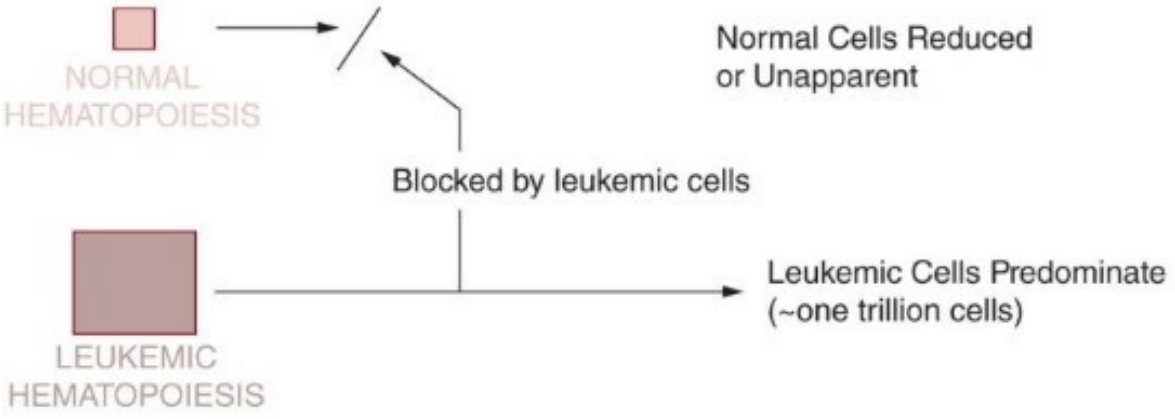
forming unit, granulocyte-macrophage [CFU-GM]) and can result in a true acute "granulocytic" leukemia without apparent intrinsic involvement of erythroid and megakaryocytic lineages.

- In t(15;17) promyelocytic AML, a subset of patients with acute monocytic leukemia, and a subset of patients with t(8;21) AML, the leukemia derives from the transformation of a granulocyte progenitor cell without intrinsic involvement of erythroid and megakaryocytic lineages.

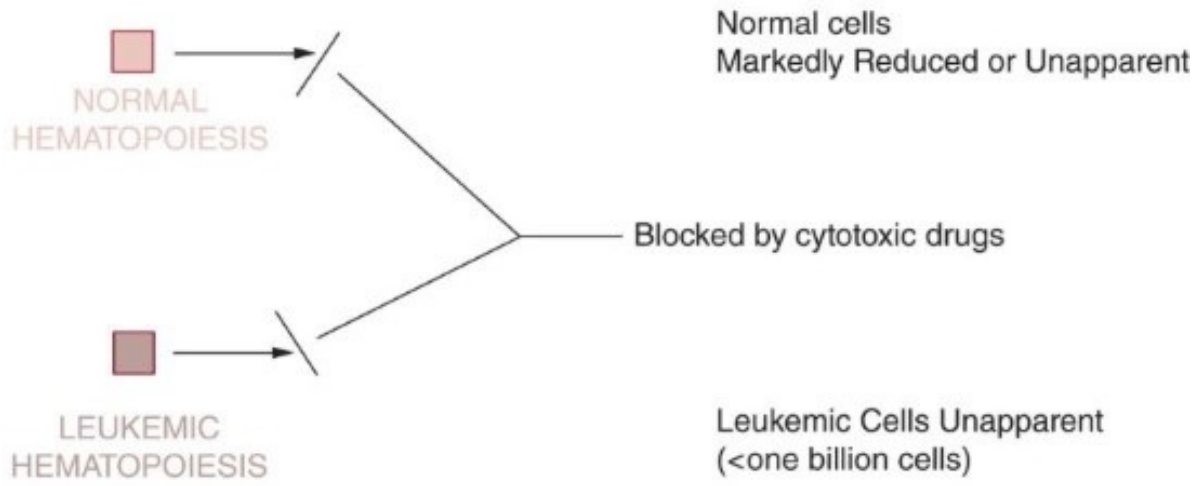
INTERPLAY OF CLONAL AND POLYCONAL HEMATOPOIESIS

- In the clonal myeloid diseases, residual polyclonal lymphohematopoietic stem cells are present in the marrow.
- These polyclonal, normal stem cells are suppressed by the effects of the malignant clone.
- These effects may be mediated by leukemic stem cells occupying or disrupting stem cell niches or by the malignant cells elaborating chemicals inhibitory to normal stem cell differentiation.
- Induction of remission in acute myelogenous leukemia involves the suppression of leukemic hematopoiesis, usually involving a reduction in leukemic cells of approximately three logs (eg, 10^{12} to 10^{9}), which would clear the marrow of microscopically evident leukemic cells. In this setting, polyclonal stem cells regain hegemony, at least for a time, and normal hematopoiesis is restored. This sequence of events defines a remission. See **Figure 40–3**.

A. LEUKEMIA IN RELAPSE



**B. LEUKEMIA
AFTER CYTOTOXIC CHEMOTHERAPY**



Iatrogenic aplastic pancytopenia provides opportunity for reemergence of normal hematopoiesis

C. LEUKEMIA IN REMISSION

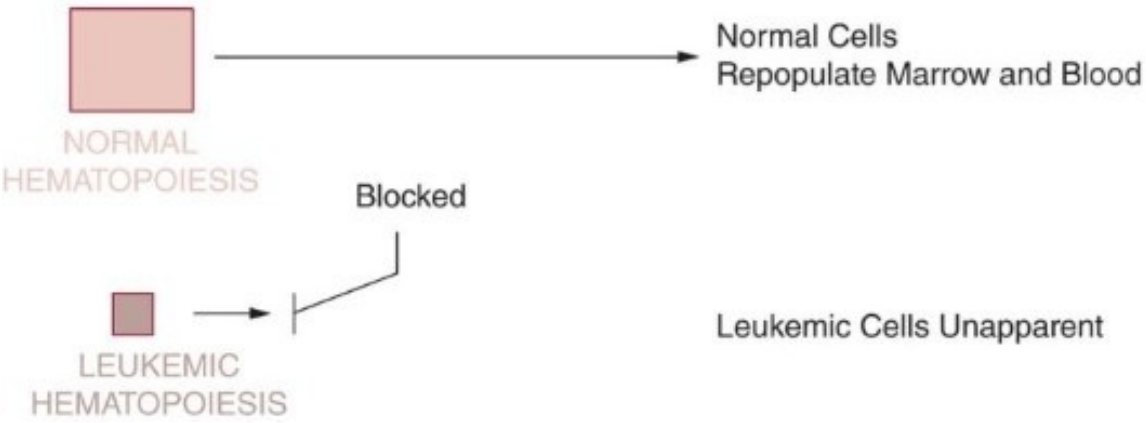


FIGURE 40–3 Remission-relapse pattern of acute myelogenous leukemia. **A.** Acute myelogenous leukemia at diagnosis or in relapse. Monoclonal leukemic hematopoiesis predominates. Normal polyclonal stem cell function is suppressed. **B.** Following effective cytotoxic treatment, leukemic cells are unapparent in marrow and blood. Severe pancytopenia exists as a result of cytotoxic therapy. The reduction in leukemic cells can release inhibition of normal polyclonal stem cell function. **C.** If reconstitution of normal hematopoiesis ensues, a remission is established and blood cells return to near normal as a result of the recovery of polyclonal hematopoiesis. This relapse-remission pattern has not been seen, generally, in the subacute and chronic myeloid leukemias treated with similar chemotherapy. Either it has not been possible to minimize the leukemic cell population with cytotoxic therapy to a point at which polyclonal hematopoiesis is restored or some other factors inhibit normal stem cell recovery. The principal exception is the effect of BCR-ABL1 inhibitor therapy in which suppression of BCR-ABL1–positive cells in chronic myelogenous leukemia can be achieved with return of polyclonal hematopoiesis. Uncommon examples of tyrosine kinase inhibitor responses in myeloid neoplasms with *pdgfr* or certain *kit* mutations may also show this pattern. In a proportion of cases, BCR-ABL1 transcripts (minimal residual disease) can be detectable along with normal, polyclonal hematopoiesis (mosaic hematopoiesis). (Reproduced with permission from Lichtman MA: Interrupting the inhibiton of normal hematopoiesis in myelogenous leukemia: a hypothetical approach to therapy. *Stem Cells* 2000;Sep;18(5):304-306.)

CLINICAL MANIFESTATIONS

Deficiency, Excess, or Dysfunction of Blood Cells

- Abnormal blood cell concentrations are the primary manifestation of clonal myeloid diseases.
- Clonal myeloid diseases may have overt qualitative abnormalities of blood cells.
- Abnormal red cell shapes, red cell enzyme activities, or red cell membrane structures may occur.
- Abnormal neutrophil granules, bizarre nuclear configurations, disorders of neutrophil chemotaxis, phagocytosis, or microbial killing may be present.
- Giant platelets, abnormal platelet granules, and disturbed platelet function may be apparent.

EFFECTS OF LEUKEMIC BLAST CELLS

Extramedullary Tumors

- Myeloid sarcomas (synonym: granulocytic sarcomas, chloromas, myeloblastomas, or monocytomas) are discrete tumors of leukemic myeloblasts or occasionally leukemic monocytes:
  - — These develop in skin and soft tissues, periosteum and bone, lymph nodes, gastrointestinal tract, pleura, gonads, urinary tract, central nervous system, and other sites.
  - — Uncommonly, they may be the first manifestation of AML, preceding the onset in marrow and blood by months or years.
  - — They are mistaken for large cell lymphomas by microscopy because of the similarity of the histopathology in biopsy specimens from soft tissues.
  - — Immunohistochemistry should be used on such lesions to identify myeloperoxidase, lysozyme, CD117, CD61, CD68/KP1, and other relevant CD markers of myeloid cells. One of four histopathologic patterns usually is evident by immunocytochemistry: myeloblastic, monoblastic, myelomonoblastic, or megakaryoblastic.
  - — They may usher in the accelerated phase of CML.
- Ph-chromosome-positive lymphoblastomas are the tissue variant of the capability of CML to transform into a terminal deoxynucleotidyl transferase-positive lymphoblastic leukemia in 25% to 30% of cases.
- Monocytomas are collections of leukemic promonocytes or monoblasts that may invade the

skin, gingiva, anal canal, lymph nodes, central nervous system, or other sites.

RELEASE OF PROCOAGULANTS AND FIBRINOLYTIC ACTIVATORS

- Hemorrhage from disseminated intravascular clotting or exaggerated fibrinolysis is a feature of acute promyelocytic leukemia.
- Hemorrhage from procoagulant-fibrinolytic state sometimes occurs in other forms of acute leukemia, especially hyperleukocytic monocytic leukemia.
- The plasma levels of protein C activity, free protein S, and antithrombin are decreased in some patients with AML, notably but not exclusively with acute promyelocytic leukemia.
- Leukemic cells may express a procoagulant tissue factor or a plasminogen activator (eg, annexin II on leukemic promyelocytes).
- Microvascular thrombosis is characteristic of the procoagulant effect. Large vessel thrombosis occurs and can result in stroke or loss of parts of extremities, but is uncommon.

HYPERLEUKOCYTIC SYNDROMES

- Five percent of patients with AML and 15% of those with CML have extraordinarily high blood leukocyte counts at diagnosis.
- In AML, leukemic cell counts over 100×10^9/L and in CML, leukemic cell counts over 300×10^9/L are usually present when the hyperleukocytic syndrome manifests itself.
- Metabolic effects (especially elevated serum uric acid and marked uricosuria) can result when massive numbers of leukemic cells in blood, marrow, and tissues are simultaneously killed by cytotoxic drugs. This can result in obstructive uropathy and renal failure.
- Leukostasis in AML and CML may be associated with effects in the pulmonary, central nervous system, special sensory, or penile circulation (**Table 40–3**).
- Sudden death can occur in patients with hyperleukocytic acute leukemia as a result of intracranial hemorrhage.
- A respiratory distress syndrome attributed to pulmonary leukostasis occurs in some patients with acute promyelocytic leukemia after all-*trans*-retinoic acid therapy. The syndrome is usually, but not always, associated with prominent neutrophilia.

TABLE 40–3 CLINICAL FEATURES OF THE HYPERLEUKOCYTIC SYNDROME

I. Pulmonary circulation
A. Tachypnea, dyspnea, cyanosis
B. Alveolar–capillary block
C. Pulmonary infiltrates
D. Postchemotherapy respiratory dysfunction
II. Predisposition to tumor lysis syndrome
III. Central nervous system circulation
A. Dizziness, slurred speech, delirium, stupor
B. Intracranial (cerebral) hemorrhage
IV. Special sensory organ circulation
A. Visual blurring
B. Papilledema

C. Diplopia
D. Tinnitus, impaired hearing
E. Retinal vein distention, retinal hemorrhages

V. Penile circulation: priapism

VI. Spurious laboratory results
A. Decreased blood partial pressure of oxygen (PO_2); increased serum potassium
B. Decreased plasma glucose; increased mean corpuscular volume, red cell count, hemoglobin, and hematocrit

Source: *Williams Hematology* 9th ed, Chap. 83, Table 83–3.

THROMBOCYTHEMIC SYNDROMES: HEMORRHAGE AND THROMBOPHILIA

- In polycythemia vera, essential thrombocythemia, and primary myelofibrosis, the height of the white cell count is a predictor of thrombosis and the height of the platelet count correlates with likelihood of bleeding (platelet count > 1000×10^9/L increases bleeding risk).
- Hemorrhagic or thrombotic episodes can occur initially or can develop during the course of thrombocythemia.
- Procoagulant factors, such as the content of platelet tissue factor and blood platelet neutrophil aggregates, are higher in patients with essential thrombocythemia than normal persons and are higher among patients with the V617F *JAK2* mutation than patients with the wild-type gene.
- Arterial vascular insufficiency and venous thrombosis are the major vascular manifestations of thrombocythemia.
- Peripheral vascular insufficiency with gangrene and cerebral vascular thrombi can occur in thrombocythemia.
- Mesenteric, hepatic, portal, or splenic venous thrombosis can develop.
- Thrombotic complications occur in about one-third of patients with polycythemia vera and, also, may occur in patients with essential thrombocythemia.
- Gastrointestinal hemorrhage and cutaneous hemorrhage, the latter especially after trauma, are most frequent, but bleeding from other sites can also occur.
- Thrombosis of the veins of the abdomen, liver, and other organs are characteristic complications in approximately half the patients with paroxysmal nocturnal hemoglobinuria.
  — Thrombosis is more common in the purely hemolytic type than in the type associated with marrow aplasia.
- A syndrome of splanchnic venous thrombosis associated with endogenous erythroid colony growth, the latter characteristic of polycythemia vera, but without blood cell count changes indicative of a myeloproliferative disease, has accounted for a high proportion of patients with apparent idiopathic hepatic or portal vein thrombosis. These cases may have blood cells with the Janus kinase 2 (*JAK2*) gene mutation without a clinically apparent myeloproliferative phenotype.
- Budd-Chiari syndrome may occur in patients with polycythemia vera or essential thrombocythemia.

SYSTEMIC SYMPTOMS

- Fever, weight loss, and malaise may occur as an early manifestation of AML.
- Fever during cytotoxic therapy, when neutrophil counts are extremely low, is nearly always a sign of infection.
- Weight loss occurs in nearly one-fifth of patients with AML at the time of diagnosis.

METABOLIC SIGNS

- Hyperuricemia and hyperuricosuria are very common manifestations of AML and CML.
- Acute gouty arthritis and hyperuricosuric nephropathy are less common.
- Saturation of the urine with uric acid accentuated by cytotoxic therapy can lead to precipitation of urate, formation of gravel or stones, and obstructive uropathy.
- Hyponatremia can occur in AML, and in some cases, is a result of inappropriate antidiuretic hormone secretion.
- Hypokalemia is commonly seen in AML.
- Hypercalcemia occurs in about 2% of patients with AML.
- Lactic acidosis has also been observed in association with AML.
- In some cases, hypophosphatemia may occur because of rapid utilization of plasma inorganic phosphate in cases of myelogenous leukemia with a high blood blast cell count and a high fraction of proliferative cells.
- Hypoxia can result from the hyperleukocytic syndrome as a consequence of pulmonary vascular leukostasis.

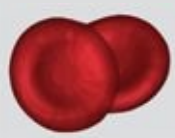
FACTITIOUS LABORATORY RESULTS

- Elevations of serum potassium levels from the release of potassium in clotted blood if there is an extreme elevation of platelets or, less often, leukocytes.
- Glucose can be falsely decreased if autoanalyzer techniques omit glycolytic inhibitors in collection tubes in cases with high leukemic cell counts.
- Factitious hypoglycemia can also occur as a result of red cell utilization of glucose in polycythemic patients.
- Large numbers of leukocytes can lower blood oxygen content spuriously as a result of its utilization in vitro during measurement.

SPECIFIC ORGAN INVOLVEMENT

- Infiltration of the larynx, central nervous system, heart, lungs, bone, joints, gastrointestinal tract, kidney, skin, or virtually any other organ may occur in AML.
- Splenic enlargement in about one-third of cases of AML and is usually slight in extent.
- Splenomegaly is present in a high proportion of cases of primary myelofibrosis (virtually 100%), CML (~80%), and polycythemia vera (~70%).
- In essential thrombocythemia, splenic enlargement is present in about 60% of patients.
  — Splenic vascular thrombi, microinfarctions, and splenic atrophy lower the frequency of splenic enlargement in thrombocythemia.

- Early satiety, left upper quadrant discomfort, splenic infarctions with painful perisplenitis, diaphragmatic pleuritis, and shoulder pain may occur in patients with splenomegaly, especially in the acute phase of CML and in primary myelofibrosis.
- In primary myelofibrosis the spleen can become enormous, occupying the left hemiabdomen.
- Blood flow through the splenic vein can be so great as to lead to portal hypertension and gastroesophageal varices. Usually, reduced hepatic venous compliance also contributes to these changes.
- Bleeding and, occasionally, encephalopathy can result from the portosystemic venous shunts in primary myelofibrosis.



For a more detailed discussion, see Marshall A. Lichtman: Classification and Clinical Manifestation of the Clonal Myeloid Diseases, Chap. 83 in *Williams Hematology*, 9th ed.

CHAPTER 41

Polycythemia Vera

- Polycythemia vera (PV) is a clonal disorder arising from somatic mutations of a multipotential hematopoietic cell, in which blood cell production, notably erythropoiesis, is increased independent of cytokine regulation. This results in exaggerated proliferation and accumulation of erythrocytic, and often also granulocytic, and megakaryocytic cells. PV is one of the myeloproliferative neoplasms (MPN), along with essential thrombocythemia (ET), primary myelofibrosis (MF), and chronic myelogenous leukemia (CML).
- Three myeloproliferative neoplasms (PV, ET, MF) share a common molecular abnormality/marker, the *JAK2* kinase V617F mutation. CML stands alone; it has a different molecular marker (bcr/abl, due to a reciprocal translocation between chromosome 9 and chromosome 22, t(9;22)(q34;q11)).

ETIOLOGY AND PATHOGENESIS

- PV arises from a neoplastic transformation of a single hematopoietic multipotential cell, which then produces a clone that suppresses and replaces normal polyclonal hematopoiesis, providing both selective growth and survival advantages to cells produced in the clone.
- The *JAK2* kinase V617F mutation directly activates erythropoietin (EPO) receptor signaling.
- In vitro erythroid colonies develop in the absence of added EPO and are characteristic for PV.
- Karyotypic abnormalities are not specific; they develop later in the disease and may portend transformation into myelofibrosis or myelogenous leukemia.
- Familial incidence of PV and/or other MPDs occurs in about 5% to 7% of patients.
- Incidence ranges from 1 to 2.5 per 100,000 reported in different countries.

CLINICAL FEATURES

- PV usually has an insidious onset, most commonly during the sixth decade of life, although may occur at any age, including childhood.
- Presenting symptoms and signs may include headache, plethora, aquagenic pruritus, thrombosis (especially Budd-Chiari syndrome), erythromelalgia, and gout. Many patients are diagnosed because of elevated hemoglobin and/or platelets on routine medical examination. Other cases may be uncovered during investigation for blood loss, iron deficiency, erythromelalgia, or thrombosis. Symptoms are reported by at least 30% of patients with polycythemia at the time of diagnosis.
- Neurologic complaints include vertigo, diplopia, scotomata, and transient ischemic events.
- Associated disorders include peptic ulcer disease and gout.

- Thrombotic and hemorrhagic events are the most common and most important complications of PV. They may occur prior to diagnosis of the disease in about one-third of patients and may be fatal. These events include stroke, myocardial infarction, deep venous thrombosis, splanchnic vein thrombosis (mostly Bud Chiari syndrome), mesenteric vein thrombosis and portal vein thrombosis, and pulmonary embolism.
- Bleeding and bruising are common complications of PV, occurring in about 25% of patients in some series (generally when platelet count is > 1000×10^9/L). Although such episodes (such as gingival bleeding, epistaxis, or easy bruising) are usually minor, serious gastrointestinal bleeding (which may mask polycythemia) and other hemorrhagic complications with fatal outcomes also can occur.
- Patients with uncontrolled PV undergoing surgery have a high risk of bleeding and/or thrombosis. Phlebotomy is recommended by many to decrease hematocrit prior to surgery to lessen risk.

LABORATORY FEATURES

- The most consistent is a mutation in exon 14 of *JAK2* kinase, present in greater than 98% of PV patients. It is a single nucleotide change $JAK2^{G1849T}$, referred to as the *JAK2* V617F mutation.
- Detection of the *JAK2* V617F mutation provides a qualitative diagnostic marker for identification of PV (as well as ET and PMF) and its differentiation from congenital and acquired reactive polycythemic disorders.
- *JAK2* V617F is present in other MPDs. In general, PV and MF patients have higher and ET patients lower *JAK2* V617F allelic burdens. In virtually all PV *JAK2* V617F-positive patients, at least some progenitors exist that are homozygous for the *JAK2* V617F mutation by uniparental disomy acquired by mitotic recombination.
- The uncommon cases of PV negative for *JAK2* V617F often carry one of a number of mutations in exon 12 of the *JAK2* gene.
- Hemoglobin is typically elevated. In patients who have or have had hepatic vein thrombosis, gastrointestinal blood loss, or have been treated with phlebotomy, hemoglobin may be normal (*masked PV*). Hypochromia, microcytosis, and other evidence of iron deficiency are often present as a result of prior chronic blood loss, usually through the stool.
- Red cell mass is elevated, implying a concomitant increase in plasma volume. However, in some patients, PV can be masked by normal hemoglobin levels.
- Nucleated red cells and teardrops (dacrocytes) are not present in the blood film.
- Absolute neutrophilia occurs in about 67% of patients. Slight basophilia also occurs in about 67% of patients.
- The platelet count is increased in more than 50% of patients and may exceed 1000×10^9/L in about 10%. Acquired von Willebrand disease due to an altered distribution and thus decreased plasma levels of von Willebrand factor may be present in patients with platelets greater than 1000×10^9/L (see Chap. 80). It is not unusual for thrombocytosis to precede elevated hemoglobin in some PV patients.
- Marrow is usually hypercellular, and iron stores may be absent. A mild degree of marrow reticulin fibrosis may be present, particularly in long-standing disease.

- Prothrombin time and partial thromboplastin time may be spuriously prolonged if the amount of anticoagulant used in the test is not adjusted for the decreased proportion of plasma.

DIAGNOSIS

- The most important diagnostic features of polycythemia vera include:
  — The presence of the *JAK2* V617F, and in a small proportion of patients, exon 12 mutations in blood cells
  — Erythrocytosis (elevated hemoglobin and or red cell mass)
  — Low serum EPO levels
  — Leukocytosis (specifically neutrophilia)
  — Thrombocytosis
  — Splenomegaly
- Other helpful clinical features are:
  — Pruritus, often provoked by a warm bath or shower
  — Elevated serum uric acid level
  — Normal or near-normal arterial oxygen saturation
- Another test of value, if available, is demonstration of erythroid colony growth in vitro in the absence of added EPO.
- Some consider the measurement of red cell mass to be the sine qua non for a diagnosis of PV, but others believe this study should be reserved for special circumstances, such as in the case of unexplained thrombocytosis, splenomegaly, or *JAK2* mutation and high normal hemoglobin concentration. If the hemoglobin concentration is greater than 16.5 g/dL in a woman or greater than 18.5 g/dL in a man, the probability of an elevated red cell mass is very high.

DIFFERENTIAL DIAGNOSIS

- The diagnostic task has been facilitated by the discovery of the *JAK2* kinase mutation that is present in greater than 98% of PV patients.
- Diagnostic criteria established by the World Health Organization (2008) are helpful in most cases (**Table 41–1**). New WHO (2014) criteria are yet to be validated by prospective clinical studies.
- *V617F*-negative patients may have PV and require a search for other *JAK2* mutations (ie, exon 12 mutations), or they may have another type of polycythemia.
- *V617*-positive patients may have another MPD.

TABLE 41–1 WORLD HEALTH ORGANIZATION CRITERIA FOR THE DIAGNOSIS OF POLYCYTHEMIA VERA (2008)

	Major Criteria	Minor Criteria
A1	Hgb > 18.5 g/dL (men) > 16.5 g/dL (women) or Hgb > 17 g/dL (men), or > 15 g/dL (women) if associated with a sustained increase of ≥ 2 g/dL from baseline that cannot be attributed to correction of iron deficiency	Marrow trilineage myeloproliferation
A2	Presence of *JAK2 V617F* or similar mutation	Subnormal serum EPO level Endogenous (EPO-independent)

	erythroid colony growth

EPO, erythropoietin; Hgb, hemoglobin.
Diagnosis satisfied if both major and one minor or first major and two minor criteria are present.
Source: *Williams Hematology*, 9th ed, Chap. 84, Table 84–1.

TREATMENT

- The mainstay of therapy for PV remains nonspecific myelosuppression, which many practitioners supplement with phlebotomies. Anagrelide may be added to control thrombocytosis (**Table 41–2**).
- Additional measures include medications to prevent thrombotic events (ie, aspirin) and to relieve symptoms. However, aspirin should not be used when platelet count is higher than 1000×10^9/L because it increases risk of bleeding.
- Promising therapies are pegylated interferon preparations, which are better tolerated than nonpegylated preparations, and JAK2 inhibitors, which are highly beneficial mainly in the post–PV-myelofibrotic stage.
- It is useful to consider treatments in the plethoric and spent phases separately.

TABLE 41–2 TREATMENT OF POLYCYTHEMIA VERA

Treatment	Advantages	Disadvantages
Phlebotomy	Low risk. Simple to perform.	Does not control thrombocytosis or leukocytosis.
Hydroxyurea	Controls leukocytosis and thrombocytosis as well as erythrocytosis.	Continuous therapy required. Long-term leukemogenic potential is not completely known.
Busulfan	Easy to administer. Prolonged remissions.	Overdose produces prolonged marrow suppression. Risks of leukemogenesis, long-term pulmonary and cutaneous toxicity.
^{32}P	Patient compliance not required. Long-term control of thrombocytosis, leukocytosis, and erythrocytosis.	Expensive and relatively inconvenient to administer. Likely leukemogenic risk.
Chlorambucil	Easy to administer. Good control of thrombocytosis and leukocytosis.	High risk of leukemogenesis.
Interferon	Low leukemogenic potential. Beneficial effect on pruritus. Potential deep suppression of the polycythemic clone.	Inconvenient to administer (injectable), costly, and adverse side effects are common.
Anagrelide	Selective effect on platelets.	Selective effect on platelets.
JAK2 Inhibitors	Decreased need for phlebotomy. Improvement in quality of life.	Recently approved, long-term benefits are unknown.

Source: *Williams Hematology*, 9th ed, Chap. 84, Table 84–3.

Plethoric Phase

- Treatment of patients in the plethoric phase of the disease is aimed at ameliorating symptoms and decreasing the risk of thrombosis or bleeding by reducing the blood counts. This is accomplished by myelosuppressive drugs and, additionally in some patients, phlebotomies, platelet-reducing agents, or pegylated interferon-α therapy.

- Treatment should be individualized according to risk factors:
  — *High risk:* patients with previous thrombosis and/or transient ischemic attacks
  — *Intermediate risk:* patients older than 60 years of age
  — *Low risk:* remaining patients
- *High-risk* and *intermediate-risk* patients require interferon or other myelosuppressive therapy.
- Absolute leukocyte count correlates with thrombotic complications; this laboratory parameter is now evaluated in the therapeutic decision-making process as are additional risk factors, including hypertension, smoking, and diabetes.
- Myelosuppression
  — Myelosuppressive therapy decreases blood counts, decreases risk of vascular events, ameliorates symptoms, and increases overall sense of well-being. Although there is also the clinical impression that it increases a patient's long-term survival, there are no clinical studies to document this.
  — At present, hydroxyurea at doses of 500 to 2500 mg daily is the preferred treatment.
  — Hydroxyurea's suppressive effect is of short duration. Thus, continuous rather than intermittent therapy is required. Because it is short acting, it is relatively safe to use; even when excessive marrow suppression occurs, the blood counts rise within a few days of decreasing the dose or stopping the drug.
  — Because it is not an alkylating agent, hydroxyurea has less potential for causing leukemic transformation.
  — Busulfan (Myleran) or P^{32} may be used in selected cases.
- Phlebotomy
  — This technique is best used in conjunction with myelosuppression; it is also used as the initial treatment by some physicians.
  — Phlebotomy alone is recommended for low-risk disease. Most patients of average size can tolerate initial phlebotomy of 450 to 500 mL about every 4 days until the target hemoglobin level is reached.
  — Phlebotomy contributes to iron deficiency. Iron supplementation is counterproductive and may result in rapid reappearance of polycythemia, but a short course of oral iron therapy is often helpful in amelioration of fatigue.
  — Alone, phlebotomy is associated with a higher incidence of thrombotic events, especially in older patients, those with a high phlebotomy requirement, and those with a prior thrombotic event.
- Summary of therapeutic approach for patients not participating in clinical trials:
  — Myelosuppression with hydroxyurea daily, both as initial therapy (1500 mg qd) and long-term treatment (500 to 2000 mg qd), aiming to maintain hemoglobin, neutrophil, and platelet counts at low-normal levels. In addition, some patients require the use of phlebotomies and/or anagrelide to maintain the hemoglobin and platelet levels in normal ranges.
  — Aspirin at a dose 80 to 100 mg daily is given to all patients without histories of major bleeding or gastric intolerance and when platelet count is not over 1000×10^9/L.
  — Allopurinol for elevated uric acid levels and medication to control pruritus are used when required.

— Judicious phlebotomies with isovolemic replacement in patients with hematocrits greater than 55% (some recommend keeping hematocrit at < 45% for men and < 43% for women) and in patients who report immediate improvement of symptoms after phlebotomy. Symptoms that may be related to hyperviscosity are headaches, difficulty concentrating, and fatigue.
— Pegylated interferon preparations are being used increasingly, with excellent results reported in pilot studies.
— JAK2 tyrosine kinase inhibitors have been shown effective and are recommended for hydroxyurea intolerant or refractory disease.

Spent Phase

- Sometimes after only a few years and usually after 10 or more years (but not in all patients), erythrocytosis in patients with PV gradually abates, phlebotomy requirements decrease and cease, and anemia develops. During this "spent" phase of the disease, marrow fibrosis becomes more marked and the spleen often becomes greatly enlarged. This condition (post-PV MF) is indistinguishable from MF (see Chap. 47). Patients typically have teardrops and leukoerythroblastic morphology, and they may have leukocytosis or leukopenia, thrombocytosis or thrombocytopenia, and immature leukocytes (including blasts) in the blood.
- Nonmyeloablative allogeneic stem cell transplantation should be considered for eligible patients as it is the only curative therapy.
- For patients not eligible for transplantation, treatment is symptomatic/supportive only. It consists of any of:
  — JAK2 inhibitors
  — Gentle myelosuppression with low doses of hydroxyurea
  — Red cell transfusion and/or erythropoiesis-stimulating agents
  — Thalidomide and its derivatives
  — Androgens
  — Experimental therapies
  — General comfort measures and analgesics
  — Splenectomy for significant cytopenias, recurrent infarctions

COURSE AND PROGNOSIS

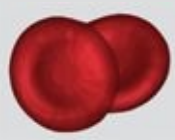
- See **Table 41–3** for criteria that define a patient's response to therapy.
- Thrombotic complications discussed in the preceding sections are the dominant cause of morbidity and mortality in patients with PV.
- In addition, and in contrast to other polycythemic disorders, PV has an increased risk of evolution to acute leukemia. While several clinical stages of PV are recognized (plethoric or proliferative phase, stable phase, spent phase or postpolycythemic myelofibrosis phase, and acute leukemia), it is not clear that these stages represent a sequential progression of the disease.
- PV is a disease that is compatible with normal or near-normal life for many years and many patients may not need any therapy, while most would benefit from aspirin. However, most

studies agree that there is excess mortality attributable to thrombotic complications and acute leukemia transformation as a direct consequence of PV.

TABLE 41–3 DEFINITION OF CLINICAL AND HEMATOLOGIC RESPONSE IN POLYCYTHEMIA VERA

Complete response: 1. Hematocrit < 45% without phlebotomy *and* 2. Platelet count ≤ 400×10^9/L *and* 3. White blood cell count ≤10×10^9/L, *and* 4. Normal spleen size on imaging, *and* 5. No disease-related symptoms
Partial response: Patients who do not fulfill the criteria for complete response, hematocrit < 45% without phlebotomy, or response in three or more of the other criteria.

Data from Barosi G, Birgegard G, Finazzi G, et al: Response criteria for essential thrombocythemia and polycythemia vera: result of a European LeukemiaNet consensus conference, *Blood*. 2009 May 14;113(20):4829-4833.



For a more detailed discussion, see Jaroslav F. Prchal and Josef T. Prchal: Polycythemia Vera, Chap. 84 in *Williams Hematology*, 9th ed.

CHAPTER 42
Essential (Primary) and Familial Thrombocythemia

- The upper limit of a normal platelet count is usually between 350 × 10^9/L and 450 × 10^9/L depending on the clinical laboratory and specific method used.
- **Table 42–1** presents the major causes of elevation of the platelet count above the normal limit.

TABLE 42–1 MAJOR CAUSES OF THROMBOCYTOSIS

Clonal thrombocytosis
Essential thrombocythemia
Polycythemia vera
Primary myelofibrosis
Chronic myeloid leukemia
Refractory anemia with ringed sideroblasts and thrombocytosis
5q-minus syndrome
Reactive (secondary) thrombocytosis
Transient thrombocytosis
Acute blood loss
Recovery from thrombocytopenia (rebound thrombocytosis)
Acute infection or inflammation
Response to exercise
Response to drugs (vincristine, epinephrine, all-*trans*-retinoic acid)
Sustained thrombocytosis
Iron deficiency
Splenectomy or congenital absence of spleen
Malignancy
Chronic infection or inflammation
Hemolytic anemia
Familial thrombocytosis
Spurious thrombocytosis
Cryoglobulinemia
Cytoplasmic fragmentation in acute leukemia
Red cell fragmentation
Bacteremia

Source: *Williams Hematology,* 9th ed, Chap. 85, Table 85–2.

ESSENTIAL THROMBOCYTHEMIA (CLONAL THROMBOCYTOSIS)

Pathophysiology

- Essential thrombocythemia (ET) is a clonal disorder of multipotential hematopoietic progenitor cell/stem cell and is a chronic myeloproliferative neoplasm related to polycythemia vera and primary myelofibrosis.
- Approximately 50% of patients express a mutant form of the *Janus (JAK)2* signaling kinase (*JAK2* V617F) found in several myeloproliferative neoplasms (polycythemia vera, primary

myelofibrosis, rare cases of myelodysplastic syndromes). The mutant allele is almost invariantly found in one copy per cell in patients with essential thrombocythemia, and leads in vivo to hematopoietic growth factor hypersensitivity, a hallmark of the disease. A very small fraction of patients display other mutations of *JAK2*.

- Mutations in calreticulin (~35% of patients) or of the thrombopoietin receptor, *Mpl* gene (~5% of patients) result in approximately 85% to 90% of the marrow and blood cells of patients with ET having one of these three gene mutations.
- Patients who do not express a mutant form of *JAK2* usually display lower hemoglobin concentrations than patients with *JAK2* V617F.

Clinical Features

- The criteria used for the diagnosis of ET are shown in **Table 42–2**.
- ET usually develops between ages 50 and 70. Sex distribution is slightly skewed toward women, especially in younger patients.
- Because platelet counts are now often done routinely, the disorder is being discovered in younger individuals and in patients who are asymptomatic.
- Rare familial cases have been reported.
- Constitutional or hypermetabolic symptoms are very uncommon.
- Mild splenomegaly is found in 40% to 50% of patients.
- Patients may have ecchymoses and bruising due to functional platelet deficiencies, or due to acquired von Willebrand disease if platelet counts are very high.
- Bleeding and thrombotic complications are major causes of morbidity and mortality. **Table 42–3** summarizes the risks of thrombosis or bleeding.
- Bleeding is common and is characteristic of platelet or vascular disorders: mucosal, gastrointestinal, cutaneous, genitourinary, and postoperative.
- Use of aspirin may occasionally lead to serious bleeding complications, especially when platelet counts are above 2000×10^9/L due to acquired von Willebrand disease.
- Thrombosis, more often arterial than venous, is most common in cerebral, peripheral, and coronary arteries.
- Twenty-five percent of all thrombotic events are lower-extremity deep venous thrombosis.
- Thrombosis in the myeloproliferative disorders often occur in unusual locations, such as hepatic artery (Budd-Chiari), sagittal venous sinus, and the upper extremity.

TABLE 42–2 DIAGNOSTIC CRITERIA FOR ESSENTIAL THROMBOCYTHEMIA

Diagnosis requires A1 to A3 or A1 + A3 to A5	
A1	Sustained platelet count > 450×10^9/L
A2	Presence of an acquired pathogenic mutation (eg, in *JAK2*, *CALR*, or *MPL*)
A3	No other myeloid malignancy, especially PV, PMF, CML, or myelodysplastic syndrome
A4	No reactive cause for thrombocytosis and normal iron stores
A5	Marrow studies showing increased megakaryocytes displaying a spectrum of morphology with prominent large hyperlobulated forms; reticulin is generally not increased

Source: *Williams Hematology,* 9th ed, Chap. 85, Table 85–1.

TABLE 42–3 RISKS OF THROMBOHEMORRHAGIC COMPLICATIONS IN ESSENTIAL THROMBOCYTHEMIA

	Thrombosis	Bleeding
Increased risk	Previous history of thrombosis	Use of aspirin and other nonsteroidal anti-inflammatory drugs
	Associated cardiovascular risk factors (especially smoking)	Extreme thrombocytosis (platelet count > 2 × 10^9/L)
	Advanced age (> 60 years)	
	Inadequate control of thrombocytosis (in high-risk patients)	
Not associated with risk	Degree of thrombocytosis In vitro platelet function	Prolonged bleeding time In vitro platelet function

Erythromelalgia and Digital Microvascular Ischemia

- This condition is caused by vascular occlusion with platelet thrombi.
- Patients have intense burning or throbbing pain, especially in the feet.
- Symptoms are exacerbated by heat, exercise, and dependency, and relieved by cold and elevation of the lower extremity.
- Painful vascular insufficiency may lead to gangrene and necrosis with normal peripheral pulses and patent major vessels on angiography.
- These problems often respond dramatically and promptly to small doses of aspirin and/or reduction of platelet count.

Cerebrovascular Ischemia

- Symptoms may be nonspecific (headache, dizziness, decreased mental acuity), and signs may be focal (transient ischemic attacks, seizures, or retinal artery occlusion).

Recurrent Abortions and Fetal Growth Retardation

- Multiple placental infarctions may lead to placental insufficiency with recurrent spontaneous abortions, fetal growth retardation, premature deliveries, and abruptio placentae.
- The use of aspirin may be necessary during pregnancy, but avoid at least 1 week prior to delivery to reduce risk of maternal or neonatal bleeding complications.

Hepatic and Portal Vein Thrombosis

- This usually occurs with polycythemia vera but may occur with essential thrombocythemia.

Blood and Marrow Findings

- Platelet counts may range from only slightly above normal to several million platelets per microliter.
- Platelets may be large, pale blue–staining, and hypogranular. Nucleated megakaryocyte fragments having a lymphoblastoid appearance may be seen occasionally in the blood film.
- Some patients may have mild leukocytosis with hemoglobin concentration ranging from normal to mild anemia.
- The leukocyte differential count is usually normal, without nucleated red cells.

- Pseudohypokalemia may occur with extreme thrombocytosis or leukocytosis.
- Marrow shows increased cellularity with megakaryocytic hyperplasia and masses of platelet debris ("platelet drifts"). Megakaryocytes are frequently giant, with increased ploidy, and occur in clusters. Significant megakaryocytic dysplasia is uncommon (**Figure 42–1**).
- Perhaps as many as one-fourth of patients with ET phenotype present without marked splenomegaly and marrow fibrosis and no blood teardrops or leukoerythroblastic morphological features. Most patients eventually evolve to primary myelofibrosis. Only expert hematopathologists can recognize their distinguishing features from ET such as megakaryocyte size variability, lobulation, and clustering. As a group, they have worse prognosis than typical ET patients.
- Some patients who have otherwise typical essential thrombocythemia will have the Philadelphia chromosome or the *BCR/ABL* gene rearrangement. They may undergo clonal evolution to a clinical phenotype most closely resembling chronic myelogenous leukemia.

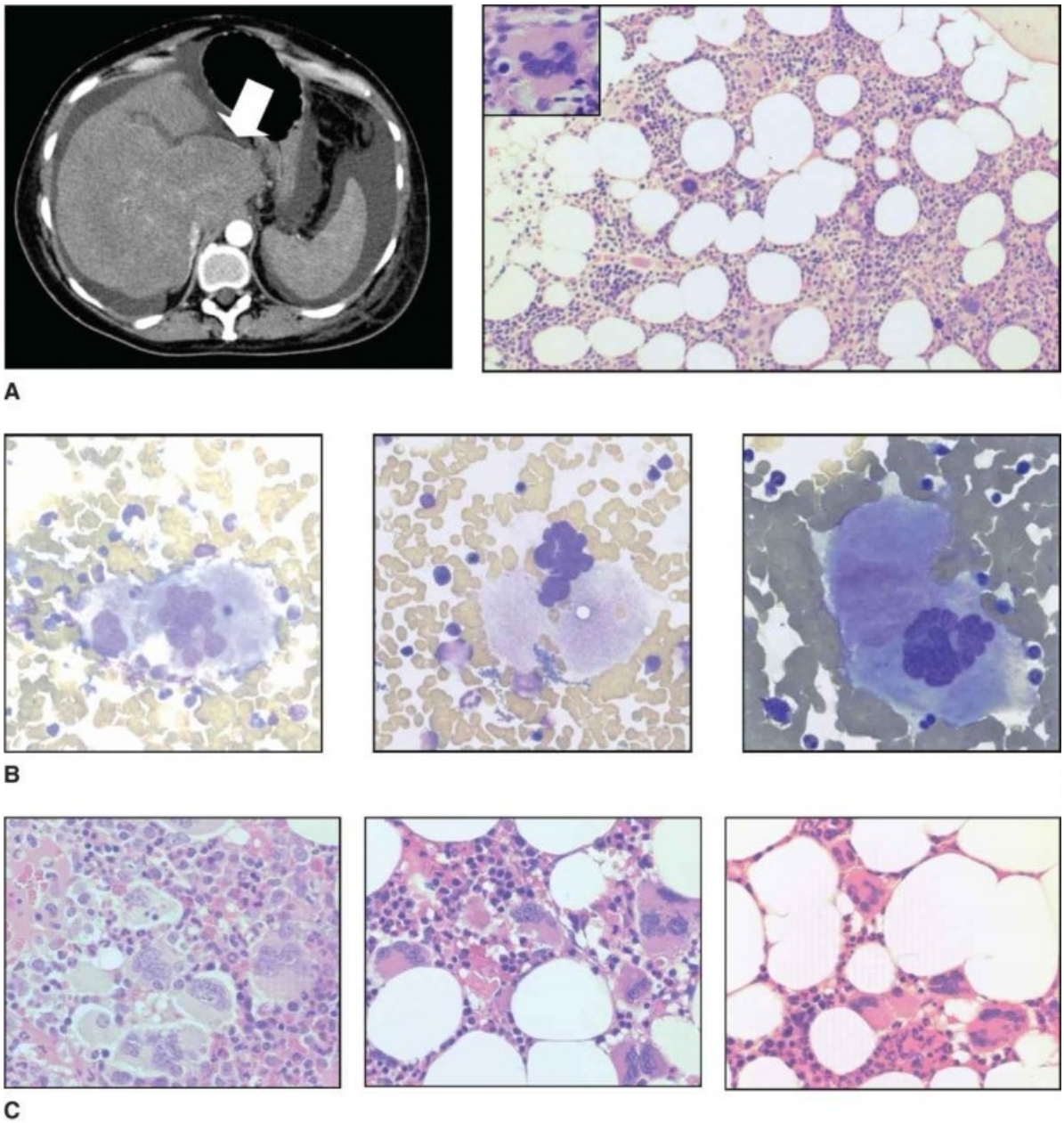


FIGURE 42–1 Morphologic features of essential thrombocythemia. **A.** Contrast-enhanced abdominal computed tomography (CT) scan showing features of established hepatic vein thrombosis in a 53-year-old woman, including hypertrophy of the caudate lobe

(arrow) with atrophy of the remaining liver and surrounding ascites; the spleen is of normal size. Hematoxylin and eosin (H&E)-stained marrow trephine biopsy showing normal cellularity and increased megakaryocytes with occasional hyperlobulated forms *(inset)*. Although the patient was *JAK2* V617F-positive, other investigations performed at this time, including blood count, red cell mass and cytogenetic analysis, were normal. **B.** Marrow aspirate from a *JAK2* V617F-positive essential thrombocythemia (ET) patient showing large, hyperlobulated megakaryocytes (slide stained with Wright-Giemsa). **C.** Marrow trephine biopsy samples from patients with ET (slide stained with H&E). (Source: *Williams Hematology*, 9th ed, Chap. 85, Fig. 85–2.)

Clinical Tests of Hemostasis

- Abnormal tests serve as a marker for the disease but do not predict bleeding and/or thrombosis and, thus, are rarely of clinical utility.
- Platelet aggregation abnormalities are variable:
  — Total loss of responsiveness to epinephrine is characteristic.
  — Reduced responses to collagen, ADP, and arachidonic acid occur in less than one-third of patients.
  — Patient platelets may display hyperaggregability or spontaneous aggregation in vitro.

Differential Diagnosis

- The diagnosis is made by genetic testing for *JAK2* V617F, another *JAK2*, *CALR*, or *Mpl* mutation, or in their absence by exclusion because there is no specific marker for the disease. The following should be demonstrated:
  — The platelet count is usually greater than 450×10^9/L, and most are greater than 600×10^9/L, on at least two occasions separated by 3 months, but occasional patients have platelet counts in the high normal range or only mildly elevated.
  — The patient is not iron deficient or afflicted by an inflammatory condition.
  — There is no other recognizable cause for secondary thrombocytosis.
  — The Philadelphia chromosome is absent.
  — There is no evidence for myelofibrosis.
  — **Figure 42–2** contains a diagnostic algorithm for patients who present with thrombocytosis.
- Patients with prefibrotic myelofibrosis (see Chap. 47) may present as ET and then progress to myelofibrosis. These patients have a worse prognosis than patients with classical ET.

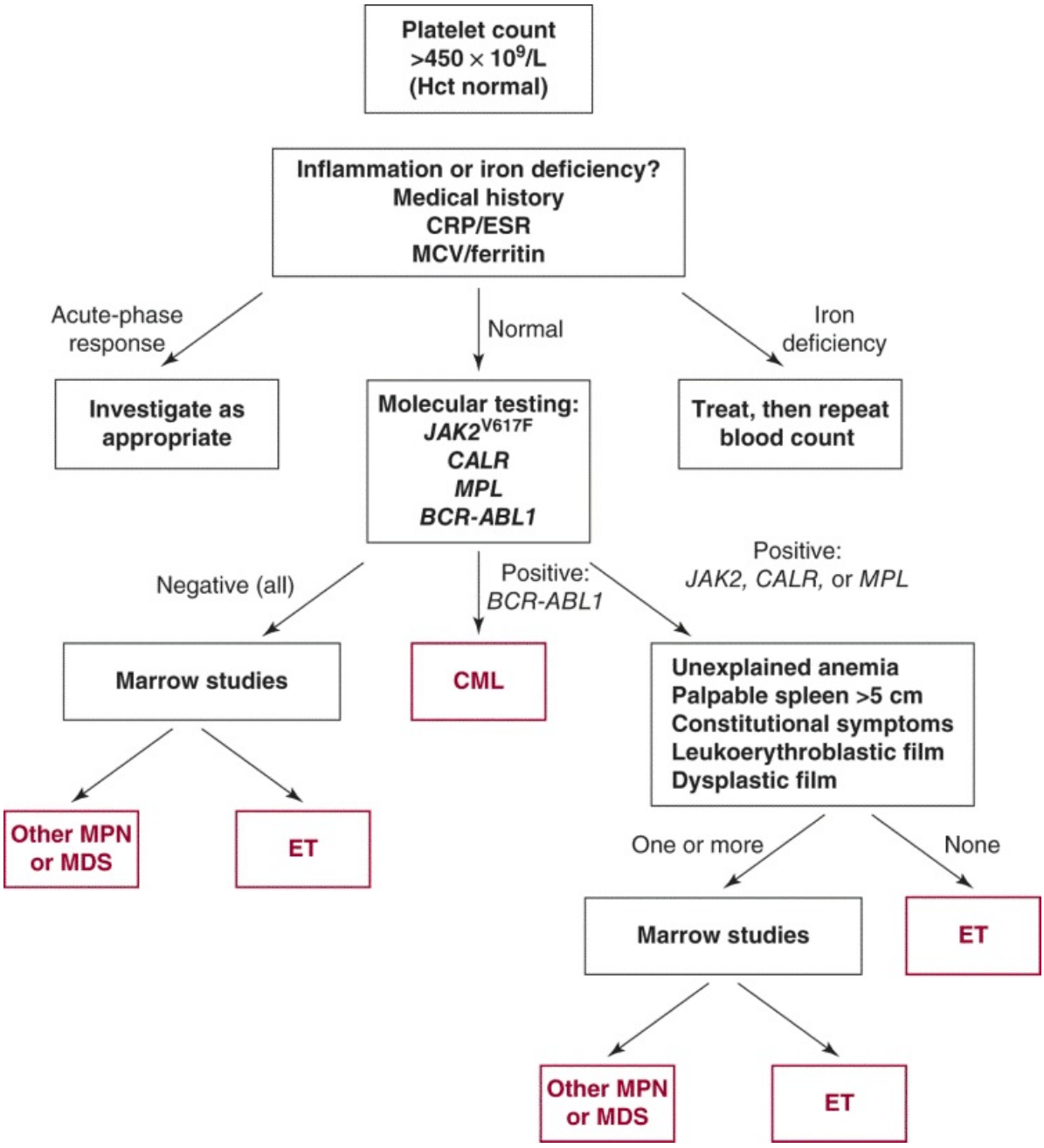


FIGURE 42–2 Investigation of patients with thrombocythemia. Algorithm outlining the investigation of a patient with an unexplained and persistently raised platelet count. CML, chronic myelogenous leukemia; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; ET, essential thrombocythemia; Hct, hematocrit; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm. (Source: *Williams Hematology*, 9th ed, Chap. 85, Fig. 85–3.)

Treatment

Asymptomatic Patients

- The need to treat asymptomatic patients is controversial.

Symptomatic Patients

- Lowering the platelet count in patients with active bleeding and/or thrombosis is beneficial.
- Prompt reduction is especially warranted in patients with microvascular digital or cerebrovascular ischemia.

Therapeutic Options

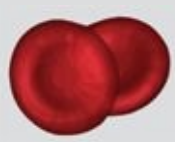
- Urgent platelet count reduction can be achieved by plateletpheresis, but the benefit is short-lived, often with a rebound increase in platelet count.
- Hydroxyurea is highly effective as initial therapy. The usual starting dose is 10 to 30 mg/kg per day orally. Blood counts should be checked within 7 days of initiating therapy and frequently thereafter, seeking a maintenance dose that will maintain the platelet count at less than 400×10^9/L. The major side effects of hydroxyurea are gastrointestinal upset and reversible painful leg ulcers, occurring in approximately 30% of patients.
- Aspirin should be added in nearly all patients requiring treatment, unless contraindicated by bleeding, allergic complications, or extremely high platelet counts.
- A large randomized study of hydroxyurea plus aspirin versus anagrelide plus aspirin demonstrated the superiority of the hydroxyurea plus aspirin arm in reducing complications.
- Anagrelide inhibits marrow megakaryocyte maturation and is effective alternative second-line therapy for patients who do not tolerate hydroxyurea. The starting dose is 0.5 mg orally four times daily or 1 mg orally twice daily. Dosage adjustments should be made weekly, depending on the blood count. The maintenance dose is usually 2.0 to 3.0 mg/d. Side effects include neurologic and gastrointestinal symptoms, palpitations, and fluid retention in approximately 2% of patients.
- Recombinant interferon-α is also effective therapy. It suppresses the abnormal megakaryocyte clone. The starting dose is 3 million units subcutaneously daily with subsequent adjustments based on tolerance and response. Two major side effects are flu-like symptoms and psychiatric disturbance, especially in older patients. It has been recommended for patients younger than 45 years of age because it is free from teratogenic or leukemogenic effects.

Course and Prognosis

- The major cause of morbidity and mortality are thrombosis and hemorrhage.
- Rarely, ET may convert to another myeloproliferative disorder, or spontaneously convert to acute leukemia.

FAMILIAL THROMBOCYTOSIS

- This rare disorder is usually inherited as an autosomal dominant trait.
- The disorder is occasionally due to mutations of the *TPO* or *c-MPL* genes, which lead to markedly increased serum thrombopoietin levels or an autonomously functioning receptor.



For a more detailed discussion, see Philip A. Beer and Anthony R. Green: Essential Thrombocythemia, Chap. 85 in *Williams Hematology*, 9th ed.

CHAPTER 43

Paroxysmal Nocturnal Hemoglobinuria

DEFINITION

- Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired hematopoietic stem/progenitor cell (HSPC) disorder characterized by deficiency of glycosyl phosphatidylinositol (GPI)-anchored proteins (GPI-APs) on the surface of hematopoietic cells. Two complement regulatory proteins (CD55 and CD59) are GPI-anchored, and deficiency of these two proteins on erythrocytes derived from the mutant HSPC leads to the complement-mediated intravascular hemolysis that is the clinical hallmark of the disease. Marrow failure and thrombophilia also complicate the disease.
- It is the only hemolytic anemia caused by an acquired (ie, somatic) mutation that is intrinsic to the red cell. The defect has its origins in the HSPC from which the circulating red cells are derived.

ETIOLOGY AND PATHOGENESIS

- PNH is a consequence of somatic mutation of *PIGA*, an X chromosome gene, that encodes a glycosyl transferase required for synthesis of the GPI anchor.
- Women and men are equally affected because *PIGA* is subject to X inactivation in somatic tissues of females. Therefore, females, like males, have only one functional *PIGA* in somatic tissues, including HSPC, that may undergo *PIGA* mutation.
- The somatic mutation arises in one or more HSPC, and as a consequence, all of the progeny of the mutant cell are deficient in all GPI-APs.
- More than 20 GPI-APs have been found to be deficient on the hematopoietic cells of PNH, but only deficiency of the complement regulatory proteins CD55 and CD59 has been shown conclusively to contribute to disease pathology.
- PNH arises as a monoclonal or oligoclonal abnormality of HSPC. Several populations of cells of different sensitivity to complement have been identified in some patients, and molecular analysis shows that the complement-sensitivity phenotype is determined by *PIGA* mutant genotype, confirming that the disease may be oligoclonal in some patients composed of PNH clones bearing different *PIGA* mutations.
- The oligoclonal nature of PNH suggests that a specific selection pressure is applied to the marrow that favors outgrowth of *PIGA* mutant HSPC present in the marrow when the selective pressure is applied. The association of PNH with aplastic anemia suggests that the selection pressure is immune-mediated. The basis of the clonal selection and clonal expansion of the *PIGA* mutant HSPC is incompletely understood.
- The extent to which the mutant clone or clones expands varies greatly among patients. In some

patients, a mutant clone may account for more than 90% of hematopoiesis, whereas in other patients, more than 10% of the blood cells are derived from a mutant clone. In general, the severity of the disease is directly related to the size of the mutant clone.

CLINICAL FEATURES

- Overt hemoglobinuria occurs irregularly in most patients, precipitated by a variety of events, including infection, surgery, trauma, and stress.
- Nocturnal hemoglobinuria is relatively uncommon as a presenting symptom.
- Patients have chronic intravascular hemolytic anemia, which may be severe, depending on the size of the mutant clone.
- Iron deficiency may be observed due to iron loss from hemoglobinuria and hemosiderinuria, as a consequence of chronic intravascular hemolysis.
- Marrow failure of varying degrees is present in all patients with PNH.
- PNH is closely associated with aplastic anemia and may be found less commonly in association with low-risk myelodysplastic syndromes.
- Bleeding may occur secondary to thrombocytopenia.
- Thrombophilia is a prominent feature and accounts for most of the mortality (see Chap. 72).
- Venous thromboses affecting unusual sites (eg, dermal veins, splanchnic vessels including Budd-Chiari syndrome, cerebral veins) is characteristic of the thrombophilia of PNH.
- Arterial thrombosis is an uncommon complication of PNH.
- Pulmonary hypertension may develop secondary to thromboses in the pulmonary microvasculature but is usually clinically inconsequential.
- Pregnancy in PNH patients may be associated with fetal and maternal complications.
- Renal manifestations include:
  — Hyposthenuria
  — Abnormal tubular function
  — Acute renal failure due to pigment nephropathy
  — Chronic renal insufficiency
- Neurologic manifestations include:
  — Headaches
  — Cerebral venous thrombosis, which may complicate PNH

LABORATORY FEATURES

- Anemia may be severe.
- Macrocytosis may be present because of mild to moderate compensatory reticulocytosis.
- The complement-mediated hemolysis of PNH is an intravascular process, and the serum lactic acid dehydrogenase (LDH) is markedly elevated in those with significant hemolysis.
- The anemia may be hypochromic and microcytic because of iron deficiency.
- Leukopenia and thrombocytopenia, as a consequence of marrow failure, are common.
- Decreased leukocyte alkaline phosphatase (LAP) activity is observed because LAP is a GPI-AP. The diagnosis of PNH is no longer based on the LAP activity but on the absence of CD55

or CD59 on blood cells.

- Marrow examination usually shows erythroid hyperplasia. The marrow may be hypocellular when PNH complicates aplastic anemia.
- Urine findings include:
  — Hemoglobinuria, its presence determined by the severity of the intravascular hemolysis
  — Hemosiderinuria, which is a constant feature in patients with significant hemolysis
- The diagnosis is made by flow cytometric analysis of GPI-linked CD59 expression on peripheral blood cells. Both erythrocytes and neutrophils should be analyzed. Analysis of the erythrocytes provides information on the phenotypes (type II, partial deficiency of GPI-AP; type III, complete deficiency of GPI-AP), and analysis of neutrophils is the best way to quantify the size of the PNH clone.

DIFFERENTIAL DIAGNOSIS

- Consider PNH in patients with pancytopenia, particularly when accompanied by evidence of intravascular hemolysis (high serum LDH, hemoglobinuria and hemosiderinuria).
- PNH should be included in the differential diagnosis of patients with thrombosis at unusual sites (eg, intra-abdominal veins), especially if there is concurrent evidence of intravascular hemolysis.
- Laboratory tests, which if abnormal in the presence of anemia or multicytopenia, may suggest a diagnosis of PNH:
  — Serum LDH concentration, reticulocyte count, analysis of iron stores, and Prussian blue stain of urine sediment for hemosiderinuria
- Definitive test:
  — Flow cytometric analysis for deficiency of CD55 and CD59 on erythrocytes and CD55, CD59 and other GPI-APs on granulocytes, which is sensitive and specific

TREATMENT

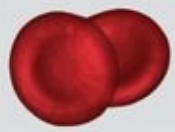
- Transfusion is used for anemia.
- Oral iron therapy is given for iron deficiency.
- Eculizumab (Soliris) is a humanized monoclonal antibody that binds to and inhibits the activation of complement C5, one of the components of the cytolytic membrane attack complex of complement. By blocking formation of the membrane attack complex, eculizumab inhibits the complement-mediated intravascular hemolysis of PNH. Eculizumab does not inhibit C3 activation on PNH erythrocytes. Consequently, the opsonized cells may be destroyed extravascularly by the mononuclear phagocyte system. Eculizumab appears safe for use during pregnancy and may reduce the risk of fetal and maternal complications.
- Steroid hormones may be useful.
  — Some patients respond to treatment with androgens. Synthetic androgens such as danazol may be preferable to naturally occurring androgens because of a more favorable toxicity profile.
  — Prednisone can be used to treat an exacerbation of the disease, but chronic use is not

recommended because of adverse effects.

- Anticoagulants may be warranted.
  — Prophylactic anticoagulation is controversial, but some studies suggest that the risk of thrombosis is related to the size of the PNH clone. For patients with clone sizes greater than or equal to 50% (based on flow cytometric analysis of expression of GPI-APs on blood neutrophils or monocytes) prophylactic anticoagulation with warfarin should be considered.
  — Anticoagulation may be useful in management of thrombotic complications. Thrombolytic therapy and/or a transjugular portosystemic shunt (TIPS) procedure should be considered for patients who develop Budd-Chiari syndrome.
- Splenectomy is rarely indicated.
- Allogeneic hematopoietic stem cell transplantation is curative. Outcomes for transplant for patients with PNH are similar to those for patients transplanted for other marrow failure syndromes.

COURSE

- Variable, but prior to eculizumab therapy most patients who were not successfully treated by hematopoietic stem cell transplantation succumbed to complications. Eculizumab appears to have favorably changed the natural history of the disease.
- Acute leukemia, aplastic anemia, or myelodysplastic syndrome may develop in some patients.



For a more detailed discussion, see Charles J. Parker: Paroxysmal Nocturnal Hemoglobinuria, Chap. 40 in *Williams Hematology*, 9th ed.

CHAPTER 44

Myelodysplastic Syndromes (Clonal Cytopenias and Oligoblastic Myelogenous Leukemia)

DEFINITION

- Myelodysplasia or myelodysplastic syndrome (MDS) is the term used, as a generalization, to encompass a diverse group of myeloid neoplasms that have in common (1) their origin in a somatically mutated multipotential hematopoietic cell, (2) ineffective hematopoiesis (late precursor apoptosis) leading to cytopenias despite a normocellular or hypercellular marrow, and (3) a propensity to undergo clonal progression to acute myelogenous leukemia (AML).
- The spectrum ranges from (1) indolent disorder with mild or moderate anemia to (2) more troublesome multicytopenias without morphologic evidence of leukemic cells to (3) oligoblastic (subacute) myelogenous leukemia with leukemic blast cells in the marrow and blood.
- Clonal cytopenia refers to disorders without increased leukemic blast cells in the marrow or blood. The manifestations vary from isolated cytopenia (eg, anemia) with a marrow with erythroid dysmorphia to severe multicytopenias with hypercellular marrow and with dysmorphia of marrow precursors in each major lineage and of blood neutrophils, red cells, and platelets.
- Because the neoplasm originates in a multipotential hematopoietic cell (eg, the lymphohematopoietic stem cell), careful inspection of blood and marrow will usually identify mild involvement of all three major lineages (eg, low normal blood cell counts) or subtle morphological abnormalities.
- Oligoblastic or subacute myelogenous leukemia (synonym: refractory anemia with excess blasts) refers to patients with cytopenias and with marrow containing 3% to 19% leukemic blast cells. (The World Health Organization [WHO] currently uses the range of 5% to 19% blast cells.)
- If the marrow blast cell count is 20% or higher, the disease is considered AML and so treated.
- The use of less than 5% marrow blasts as a demarcation between a normal and pathological blast percentage is an anachronism dating from a decision made in 1955, at which time the first definition of a remission in childhood acute lymphoblastic leukemia used less than 5% marrow blasts (and other salutary changes) to avoid additional cytotoxic treatment in an era without platelet transfusion, potent wide-spectrum antibiotics, or other support for children treated with multidrug regimens. Also, only light microscopy was available to distinguish nonleukemic lymphocytes in treated marrows from residual leukemic lymphoblasts. This so-called “5 percent rule,” however, is not relevant at the time of diagnosis, especially in the case of myeloid neoplasms, has not been validated, but it has been ensconced.
- The use of less than or greater than or equal to 20% blasts to distinguish oligoblastic

myelogenous leukemia (refractory anemia with excess blasts) from AML is arbitrary and without pathobiologic foundation. Studies have shown no difference in phenotypic manifestations or survival between populations with 10% to 19% as compared to 20% to 30% leukemic myeloblasts. The physician should determine management of the case by several factors (eg, physiologic age, severity of cytopenias, cytogenetic or oncogene risk category, transfusion requirements, frequency and severity of infections), not principally whether a patient with myelogenous leukemia has 10%, 15%, 20%, or 25% blast cells.

- More rarefied diagnostic categories are in the WHO classification shown in **Table 44–1** and are currently being revised. These categories are useful in ensuring comparable populations in therapeutic or prognostic clinical trails. In individual patients, management is best assessed by the clinical manifestations and clinical setting of the patient under one's care and the risk of progression as judged by factors discussed below under "Risk Categories."

TABLE 44–1 **WORLD HEALTH ORGANIZATION CLASSIFICATION OF THE MYELODYSPLASTIC SYNDROMES**

1. Refractory cytopenia with unilineage dysplasia (RCUD)
   Dysplasia in ≥10% of cells from a single myeloid lineage
   < 5% marrow blasts, < 1% blood blasts, and no Auer rods
   < 15% of erythroid precursors are ring sideroblasts

Most often is refractory anemia (RA) but can be refractory neutropenia (RN) or refractory thrombocytopenia (RT) in rare cases

2. Refractory anemia with ring sideroblasts (RARS)
   Isolated erythroid dysplasia
   < 5% marrow blasts, < 1% blood blasts, and no Auer rods
   ≥15% of erythroid precursors are ring sideroblasts

The cutoff for ring sideroblasts is arbitrary and does not reflect the clinical behavior of this subtype as accurately as the frequently associated mutations of SF3B1

3. MDS associated with isolated del(5q)
   5q31 deletion as the sole chromosomal abnormality
   Normal to increased megakaryocytes with hypolobated nuclei
   Normal to increase platelet count
   < 5% marrow blasts, < 1% blood blasts, and no Auer rods

This subtype overlaps with, but is not entirely synonymous with the "5q-minus syndrome" recognized prior to the establishment of the WHO classification system for MDS

4. Refractory cytopenia with multilineage dysplasia (RCMD)
   Dysplasia in ≥10% of cells from two or more myeloid lineages
   < 5% marrow blasts, < 1% blood blasts, and no Auer rods
   Blood monocyte count < 1×10^9/L
5. Refractory anemia with excess blasts (RAEB)
   Type 1: 5%–9% marrow blasts, < 5% blood blasts, *and* no Auer rods
   Type 2: 10%–19% marrow blasts, 5%–19% blood blasts, *or* Auer rods
   Blood monocyte count < 1×10^9/L
6. Unclassifiable MDS (MDS-U)
   Minimal dysplasia in the presence of a clonal cytogenetic lesion considered presumptive evidence of MDS
   < 5% marrow blasts, < 1% blood blasts, and no Auer rods

Source: *Williams Hematology,* 9th ed, Chap. 87, Table 87–1.

ETIOLOGY AND PATHOGENESIS

- The fundamental alteration is a somatically mutated lymphohematopoietic stem cell or a very closely related multipotential progenitor, resulting in trilineage blood cell abnormalities in

most cases. Even in clonal anemia in which the neutrophils count and platelet count are within the normal range, evidence of dysmorphia in neutrophil and megakaryocyte-platelet lineages may be evident on careful examination.

- Epigenetic modification contributes to the hematopoietic abnormalities and is a target for therapy.
- Overt cytogenetic abnormalities are found in approximately 15% of patients with clonal anemia, but these abnormalities increase in prevalence to about 70% in patients with oligoblastic myelogenous leukemia. Overall, approximately 50% of patients have an overt chromosome abnormality.
- DNA-damaging chemotherapeutic agents (especially alkylating agents and topoisomerase II inhibitors) or high-dose radiation can increase the risk of developing myelodysplasia (as well as AML). High-dose, prolonged benzene exposure, rare in countries with workplace regulations, and long-standing tobacco smoking are external risk factors. Obesity may be an endogenous risk factor.
- Exaggerated apoptosis of late hematopoietic precursors results in blood cytopenias in the face of a hypercellular marrow.
- A very small fraction of patients (approximately 5%) may have hypocellular marrows akin to the similar small fraction with hypocellular AML.

PREVALENCE OF MUTATIONS IN CELLS OF PATIENTS WITH MYELODYSPLASIA

- Mutation of *SF3B1* (encodes a splicing factor) is present in approximately 2% of patients (Table 44–2). It is the only somatic mutation in MDS patients that confers a favorable prognosis (longer overall survival and lower frequency of clonal evolution to AML). This mutation has a predictive value of 98% for the presence of ringed sideroblasts in the marrow.
- Mutation of *JAK2* is associated with clonal anemia with ringed sideroblasts and thrombocytosis.
- Mutation of TP53 is associated with complex karyotypes and a less favorable prognosis.

TABLE 44–2 RECURRENTLY MUTATED MYELODYSPLASTIC SYNDROME GENES

	Mutated Gene	Frequency in MDS (%)	Prognostic Value	Additional Information
Splicing	*SF3B1*	20–30	Favorable	Strongly associated with ring sideroblasts
	SRSF2	10–15	Adverse	More frequent in CMML
	U2AF1	8–12	Adverse	Associated with del(20q)
	ZRSR2	5–10	?	
Epigenetic regulators	*TET2*	20–25	Neutral	More frequent in CMML
	DNMT3A	12–18	Adverse	
	IDH1/IDH2	< 5	?	
	ASXL1	15–25	Adverse	More frequent in CMML
	EZH2	5–10	Adverse	More frequent in CMML

	ATRX	< 2	?	Associated with ATMDS
	KMD6A	< 2	?	
Transcription	*RUNX1*	10–15	Adverse	Familial in rare cases
	GATA2	< 2	?	Commonly familial, rarely somatic
	ETV6	< 5	Adverse	Rarely translocated in MDS
	PHF6	< 2	?	
	TP53	8–12	Adverse	Associated with complex karyotypes
Cohesins	*STAG2*	5–10	?	
	RAD21	< 5	?	
	SMC3	< 2	?	
	SMC1A	< 2	?	
Signaling	*NRAS/KRAS*	5–10	Adverse	More frequent in CMML
	JAK2	< 5	Neutral	Enriched in RARS-T
	CBL/CBLB	< 5	Adverse	More frequent in CMML
	PTPN11	< 2	Adverse	More frequent in JMML, can be germline
Others	*GNAS/GNB1*	< 2	?	G-protein signaling pathway
	BRCC3	< 2	?	DNA repair pathway
	PIGA	< 2	?	Cause of PNH clones
	TERT/TERC	< 2	?	Can be germline
	FANC genes	< 2	?	Typically germline

Source: *Williams Hematology*, 9th ed, Chap. 87, Table 87–2.

EPIDEMIOLOGY

- Incidence increases exponentially from age 40 (0.2/100,000 persons) to age 85 years (45 per 100,000 persons) in the United States (**Figure 44–1**).
- In younger adults, the onset is often preceded by chemotherapy or irradiation (treatment-related myelodysplasia) for another neoplasm (eg, breast, ovary) or a severe autoimmune disease.
- Male-to-female ratio is about 1.5:1 (except in 5q- syndrome, in which females predominate).
- Children 0.5 to 15 years have an incidence rate of 0.1 per 100,000/year.
- Children usually have more advanced types (oligoblastic myelogenous leukemia).
- Childhood cases may evolve from predisposing inherited syndromes such as Fanconi anemia.

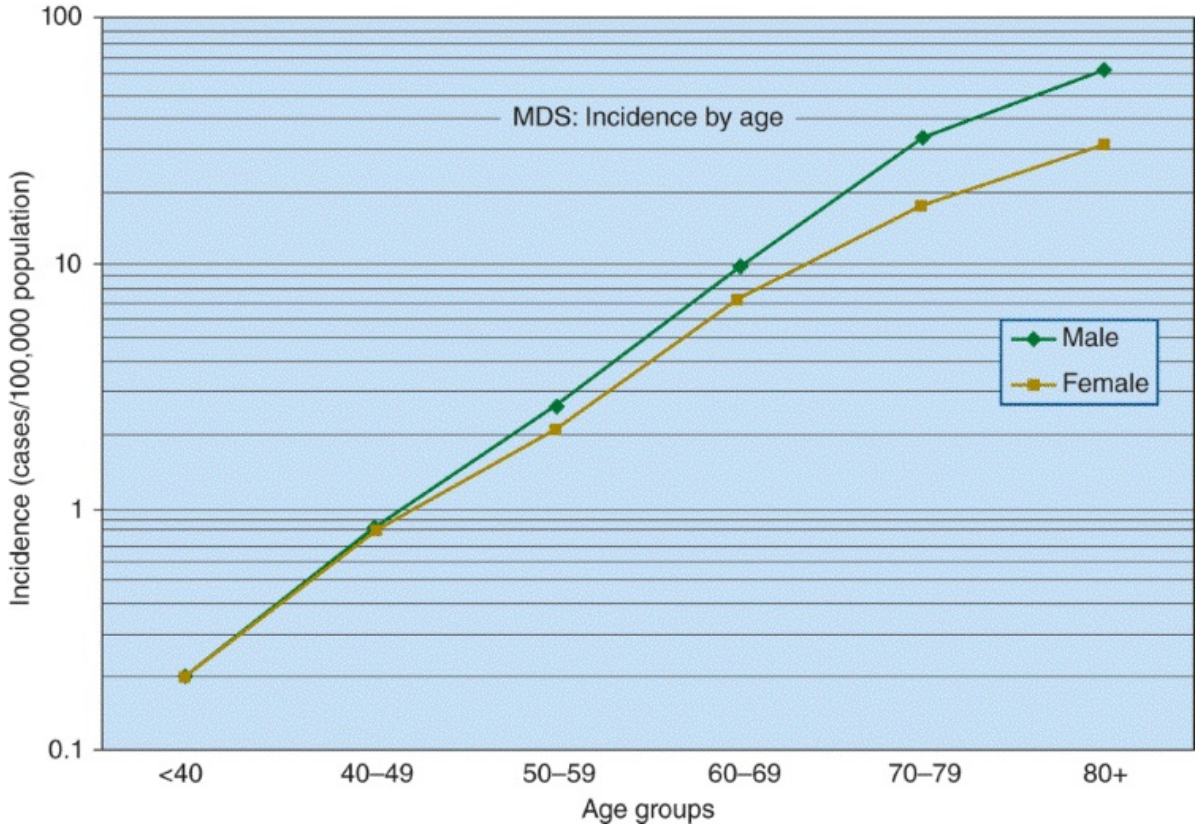


FIGURE 44–1 The annual incidence of myelodysplastic syndrome shown by age. There is an exponential (approximately linear on semilogarithmic plot) increase in incidence from age 40 years on. In persons younger than age 40 years, the incidence is so low that it is aggregated (< 40). (Data from the United States National Cancer Institute, Surveillance, Epidemiology, and End-Results Program.)

CLINICAL FEATURES

- Disease may be asymptomatic if mild anemia is the principal feature, with little or no reductions in platelet and neutrophil counts.
- If moderate or severe anemia and/or neutropenia and thrombocytopenia develop, loss of sense of well-being, pallor, dyspnea on exertion, easy bruising, and slow healing of minor cuts may be evident. The presence and intensity of these manifestations are a function of the gradient from mild anemia to severe pancytopenia.
- Hepatomegaly or splenomegaly are uncommon findings (< 10%).
- Hypothalamic malfunction with loss of libido and diabetes insipidus, neutrophilic dermatosis (Sweet syndrome), and inflammatory syndromes mimicking lupus erythematosus are each rare associated findings.

LABORATORY FEATURES

- Anemia occurs in more than 85% of patients and may be macrocytic, with rare circulating nucleated red cells. Misshapen cells (eg, elliptocytes, other poikilocytes), anisochromia, and basophilic stippling are hallmarks of the red cell dysmorphia in the blood film (see **Figure 44–2**).
- Hemoglobin F levels may be increased; hemoglobin H may be present, rarely (with red cell

shape and inclusions simulating α-thalassemia); and red cell enzyme activities may be abnormal.

- Neutropenia occurs in at least 50% of cases. Coarse chromatin, nuclear hyposegmentation (acquired Pelger-Huèt abnormality), and decreased cytoplasmic granulation of neutrophils commonly occur.
- Monocytosis often found and rarely may be the principal abnormality.
- Thrombocytopenia or occasionally thrombocytosis may be present. The latter is especially notable in the 5q- syndrome. Platelets may be large, with decreased or fused granules. Platelet aggregation tests may be abnormal. Micromegakaryocytes may enter the blood.
- Marrow abnormalities include (1) hypercellularity, (2) delayed nuclear maturation of red cell precursors, (3) abnormal cytoplasmic maturation of red cell and neutrophil precursors, (4) pathologic sideroblasts (eg, ringed sideroblasts), (5) megakaryocytes with unilobed or bilobed nuclei or odd number of nuclear lobes, (6) micromegakaryocytes, and (7) an increased fraction of myeloblasts. In approximately 5% of patients, the marrow may be hypocellular, simulating aplastic anemia. Careful search may show unequivocal clusters of dysmorphic hematopoietic precursors and cytogenetic analysis may uncover a clonal abnormality, either or both indicative of myelodysplasia. Immunophenotyping to rule out paroxysmal nocturnal hemoglobinuria should be done in this setting (see Chap. 43).
- Chromosomal abnormalities occur in up to 70% of patients with oligoblastic myelogenous leukemia by G-banding or fluorescent in situ hybridization. The most common abnormalities (very similar to those in AML) are del(5q), -7/del(7q), trisomy 8, -18/del(18q), and del(20q) but innumerable other less common clonal cytogenetic changes may be present as may complex abnormalities (more than three abnormalities).
  - — del(5q) occurs in approximately 15% of patients. Small deletions in 5q32-33.3 are associated with a favorable prognosis and with increased sensitivity to lenalidomide. Sole del(5q) in this region is the only genetically defined subtype of MDS. Some may have "5q minus syndrome" (see "5q- Syndrome," below). Larger or different deleted areas on 5q in patients with MDS may confer a poorer risk.
  - — Monosomy 7 or del(7q) found in approximately 5% of patients has adverse prognostic import.
  - — Monosomy 17 or del(17p) has an adverse prognosis and may be associated with TP53 mutation.
- Occasional patients with thrombocytosis may have an abnormality of chromosome 3.
- Common translocations such as t(8;21), t(15;17), or t(16;16), seen in AML, especially in younger patients, are not features of the cells in MDS.
- Ferritin levels are often increased at diagnosis as a result of a shift in erythron iron to the storage compartments in proportion to the degree of anemia and as a result of increased iron absorption.

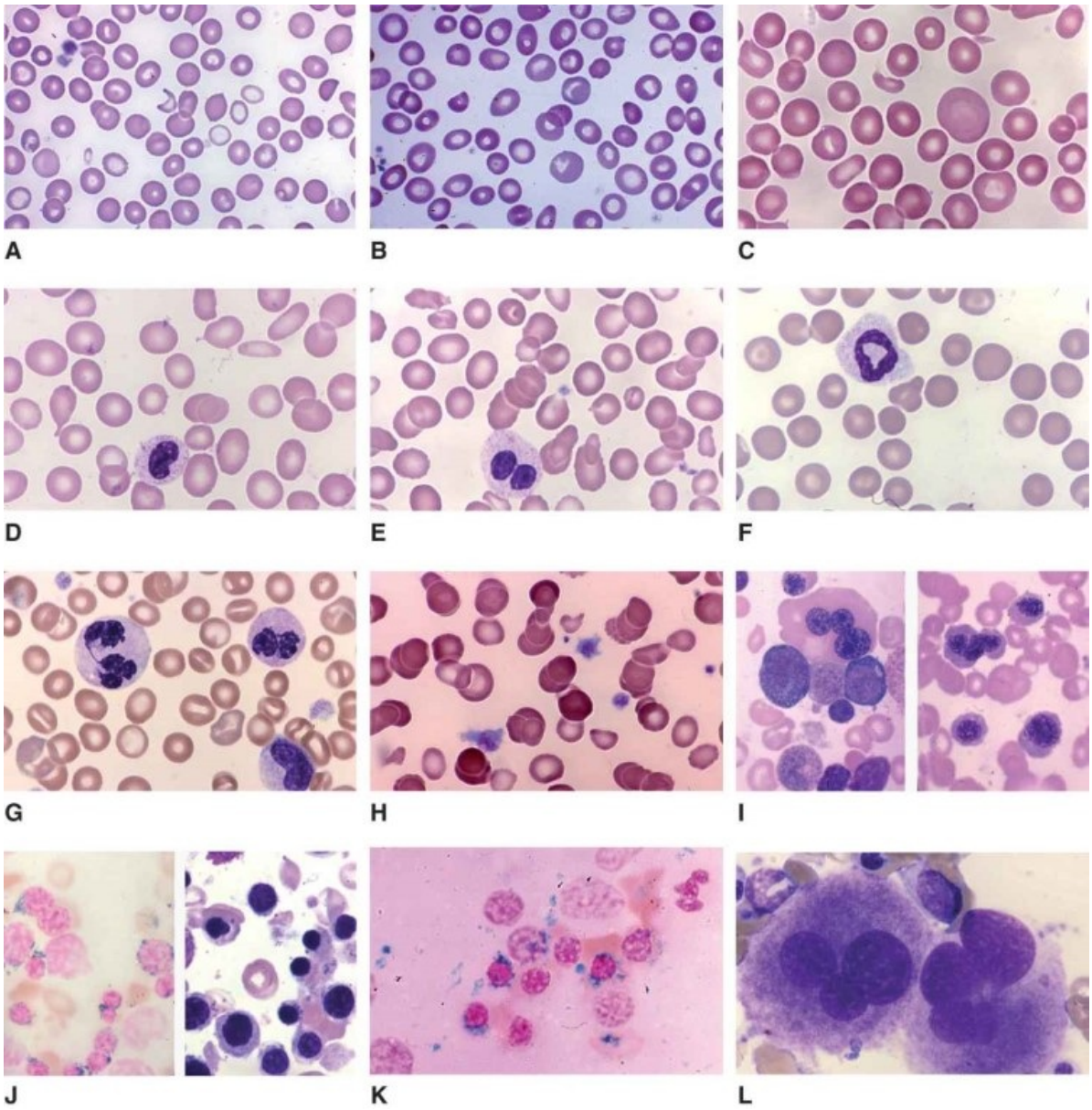


FIGURE 44–2 Blood and marrow films from patients with clonal cytopenias (MDS). **A.** Blood film. Anisocytosis. Poikilocytosis with occasional fragmented cells. Marked anisochromia with marked hypochromia, mild hypochromia and normochromic cells. **B.** Blood film. Marked anisocytosis. Mild anisochromia. Poikilocytes with occasional fragmented cells and oval and elliptical cells. Two polychromatophilic macrocytes. **C.** Blood film. Striking anisocytosis with giant macrocytes and microcytes. Poikilocytes with tiny red cell fragment and elliptocyte. **D.** Blood film. Mild anisocytosis. Ovalocytes and elliptocytes. Dacryocyte. Hyposegmented neutrophil with poor cytoplasmic granulation. **E.** Blood film. Marked anisocytosis (macrocytes and microcytes). Ovalocytes and elliptocytes. Acquired Pelger-Huèt nuclear anomaly (classic pince-nez shape) in neutrophil. **F.** Blood film. Mild anisocytosis. Abnormal neutrophil with ring nucleus. **G.** Blood film. Anisochromia. Stomatocytes. Abnormal neutrophil nuclei with hyperlobulation and hyperchromatic staining. Note abnormal elongated nuclear bridge in neutrophil on left. **H.** Blood film. Atypical platelets. Two macrothrombocytes with excess cytoplasm and atypical central granules. Anisocytosis (conspicuous microcytes). Anisochromia (conspicuous hypochromic cells). Poikilocytosis with occasional fragmented red cells. **I.** Marrow film. Wright stain. Trilobed megakaryocyte. Wright stain. Macroerythroblasts. **J.** Marrow films. Prussian blue stain. Ringed sideroblasts. Wright stain. Erythroid hyperplasia with macroerythroblasts. **K.** Marrow film. Prussian blue stain. Ringed sideroblasts. **L.** Marrow film. Wright stain. Trilobed megakaryocytes. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

DIAGNOSTIC CRITERIA FOR MYELODYSPLASTIC SYNDROME

- **Table 44–3** contains guidelines for arriving at a diagnosis of an MDS.

TABLE 44–3 **DIAGNOSTIC CRITERIA FOR MYELODYSPLASTIC SYNDROMES**

Presence of one or more otherwise unexplained cytopenias*		
Hemoglobin < 11 g/dL		
Absolute neutrophil count < 1500/μL		
Platelet count < 100,000/μL		
Presence of one or more MDS decisive criteria		
> 10% dysplastic cells in erythroid, myeloid, and/or megakaryocyte lineages		
5% to 19% marrow blasts		
Evidence of a cytogenetic abnormality typical for MDS:		
–7 or del(7q)	del(12p) or t(12p)	t(1;3)(p36.3;q21.1)
–5 or del(5q)	del(9q)	t(2;11)(p21;q23)
i(17q) or t(17p)	idic(X)(q13)	inv(3)(q21;q26.2)
–13 or del(13q)	t(11;16)(q23;p13.3)	t(6;9)(p23;q34)
del(11q)	t(3;21)(q26.2;q22.1)	
Exclusion of alternative diagnosis that explain blood and marrow findings		
No AML-defining criteria (eg, t[8;11], i[16], t[16;16], t[15:17], or erythroleukemia)		
No other hematologic disorders (eg, acute lymphocytic leukemia, aplastic anemia, or various lymphomas)		
Not explained by		
HIV or other viral infection		
Deficiencies of iron or copper		
B_{12}, folate, or other vitamin deficiency		
Medications (eg, methotrexate, azathioprine, or chemotherapy)		
Alcohol abuse (typically heavy and prolonged usage)		
Autoimmune conditions (eg, immune thrombocytopenia purpura, immune hemolytic anemia, Evans syndrome, Felty syndrome, or systemic lupus erythematosus)		
Congenital disorders (eg, Fanconi anemia, Diamond-Blackfan anemia, and Shwachman-Diamond syndrome)		

*Present for 6 months or longer, if there is no typical cytogenetic abnormality identified.
Source: *Williams Hematology*, 9th ed, Chap. 87, Table 87–3.

SPECIFIC SYNDROMES

Clonal (Refractory) Sideroblastic Anemia

Clinical and Laboratory Features

- Most patients are older than 50 years of age and have anemia.
- Anemia may be mild to severe.
- Macrocytosis with anisochromia (hypochromia and normochromia) of red cells is common.
- Basophilic stippling of red cells and anisocytosis are common.
- Reticulocyte response is inadequate to degree of anemia.
- Ineffective erythropoiesis with impaired heme synthesis and mitochondrial iron overload are characteristic findings.
- Approximately 90% of patients have hematopoietic cells containing the *SF3B1* mutation.
- Marrow cellularity is increased, with defective cytoplasmic maturation of erythroblasts, and ringed sideroblasts are present.
- Serum iron, ferritin levels, and saturation of transferrin are increased. Prussian blue stain of marrow shows increased storage iron.
- A smaller proportion of patients may have overtly abnormal granulopoiesis or megakaryocytopoiesis.
- Neutropenia or thrombocytopenia present in a modest proportion and may not be consequential

functionally.

Treatment

- Treatment options are predicated on specific abnormalities and prognostic category (see “Risk Categories” section below).
- The same treatment options are available for patients in any subtype of myelodysplasia depending on morbid manifestations.
- The main considerations in all subtypes are (1) severity of anemia (demethylating agents, erythropoietic stimulants), (2) severity of neutropenia and infections (antibiotics), (3) severity of thrombocytopenia and evidence of bleeding (platelet transfusion), (4) special case of hypoplastic marrow (immunosuppressive therapy), and (5) evidence of progressive leukemic hematopoiesis (blast level) requiring acute leukemia type cytotoxic therapy or allogeneic hematopoietic stem cell transplantation. See following details.
- Asymptomatic patients without evidence of progression may not require treatment.
- Disorder may not progress for many years.
- Folic acid (1 mg/d orally) should be used if serum and red cell folic acid content are low.
- Pyridoxine (200 mg/d orally) can be tried if sideroblastic anemia is symptomatic, but success is rare.
- Danazol (200 to 400 mg/d orally) has occasionally increased platelet count or decreased frequency of platelet transfusion.
- In uncommon patients with clonal cytopenia and hypoplastic marrows, cyclosporine and antithymocyte globulin have resulted in improvement in hematopoiesis (see Chap. 3).
- Human recombinant erythropoietin (EPO) coupled with granulocyte colony-stimulating factor (G-CSF) may improve moderately severe anemia or may markedly decrease transfusion requirements. This combination is usually not beneficial unless (1) the serum EPO level is less than 500 units/L and (2) the transfusion requirement is less than 2 units of red cells per month.
- 5-Azacytidine, 75 mg/m^2 per day, subcutaneously (or intravenously) for 7 days every 28 days can be useful in patients with symptomatic anemia and requirement for frequent transfusions. About 40% of patients have a very good response as judged by the increase in hemoglobin levels or a significant decrease in transfusion frequency. Heretofore, the drug was used in patients failing to respond to EPO and G-CSF but evidence that its use may delay progression of the disease and prolong survival in responders is likely to make it a first choice in such patients, administered before the results of EPO + G-CSF are evident. Although 5-azacytidine is an antimetabolite, its benefit is thought to be related principally to its demethylating effects (reversing deleterious epigenetic effects).
- 5-Azacytidine may initially cause a fall in blood cell counts, even in responders, and transfusion requirements may increase during the first one to several weeks of therapy. It may take four to five cycles of therapy to achieve a response and maximum responses may not occur for nine to ten cycles.
- Decitabine, another cytotoxic agent with demethylating effects, has been approved for use in lieu of 5-azacytadine. One regimen proposed is 20 mg/m^2 intravenously over 1 hour daily for 5 days to be repeated every 4 weeks for a total of three cycles. Worsening of cytopenias may occur initially, but some experts recommend that unless a complication of cytopenia develops or other serious side effects occur, the treatment program should not be modified.

- Red cell transfusion may be necessary if severe anemia or if coincidental ischemic diseases (eg, angina or heart failure) are present with moderate anemia.
- Some patients with high red cell transfusion requirements develop iron overload and should receive iron chelation therapy. Oral deferasirox, 20 mg/kg per day, has been approved by the US Food and Drug Administration for this purpose. It can produce nausea, vomiting, abdominal pain, skin rash, and other adverse effects (see Chap. 9).
- One guideline for use of iron chelator therapy requires three criteria to be met: (1) probable survival for at least 1 year, (2) receipt of more than 20 units of red cells, and (3) a serum ferritin of greater than 1000 µg/L.
- Some patients do not progress or may live for many years before progression occurs. Others may have worsening hematopoiesis, more severe cytopenias, and morbidity and mortality from recurrent severe infections or hemorrhage.
- On average, a normal or only slightly abnormal neutrophil and platelet count suggests a better prognosis. A mild anemia not requiring transfusions also portends a better outcome on average.
- About 10% of patients progress to AML over a 10-year period of observation.
- Median survival is about 7 to 9 years in different studies.

Clonal (Refractory) Nonsideroblastic Anemia

- This is similar to refractory sideroblastic anemia in all respects but without or with rare pathologic sideroblasts in the marrow. (The WHO currently defines this category as having less than or equal to 15% ringed [pathologic] sideroblasts; however, a proportion of patients carry the *SF3B1* mutation.) Given the data accruing on the relationship of *SF3B1* mutation and marrow sideroblasts, the WHO will probably reclassify this disorder to be aligned with the absence of the *SF3B1* mutation and not related to the percent marrow sideroblasts. This change would probably reduce the frequency of sideroblasts in this category to nil (more properly aligned with its designation).

Clonal Multicytopenia with Hypercellular Marrow

- These patients present with clonal cytopenias with neutropenia and/or thrombocytopenia, as well as anemia.
- The blood and marrow findings are as described for clonal anemia, although neutropenia and/or thrombocytopenia are more prominent. Dysmorphic neutrophils (acquired Pelger-Huèt nuclear and/or granule abnormalities) and platelets (giant size, abnormal granulation) may be evident in the blood. Dysmorphic neutrophil precursors and megakaryocytes with nuclear and cytoplasmic abnormalities are usually evident (eg, abnormal neutrophil precursor granulation and nuclear hyposegmentation or hypersegmentation, hypolobulated or hyperlobulated megakaryocyte nuclei, micromegakaryocytes).
- Marrow and blood picture can be seen in patients with the acquired immunodeficiency syndrome, but progression to acute leukemia is not a feature.
- Fever, especially in the setting of neutropenia, should be evaluated promptly and a suspected infection treated with broad-spectrum antibiotics, unless and until culture results permit more specific therapy.

- Therapy is as described in more detail in the section "Clonal (Refractory) Sideroblastic Anemia: Treatment" above. One or more of the following may be required: (1) EPO and G-CSF, (2) 5-azacytidine or decitabine, and (3) red cell and platelet transfusions.
- In patients with severe morbidity and a suitable donor, allogeneic hematopoietic stem cell transplantation may be considered (using either myeloablative or nonmyeloablative conditioning depending on patient age, comorbid conditions, and other individual factors).
- Median survival is about 2.5 to 4 years in different studies. AML develops in about 50% of patients, and about 25% die of infection or hemorrhage.
- Patients with complex cytogenetic patterns have a worse prognosis.

The 5q– Syndrome

- Patients have a refractory anemia, with marrow abnormalities of dyserythropoiesis; erythroid multinuclearity; and hypolobulated, small megakaryocytes (see **Figure 44–3**).
- Most patients do not have consequential neutropenia or thrombocytopenia.
- A proportion of patients have thrombocytosis (> 450 × 10^9/L). About 40% of patients with clonal anemia and thrombocytosis have hematopoietic cells carrying the *Janus Kinase 2* (*JAK 2*) mutation. This variant has a favorable prognosis.
- The syndrome is most common in older women, but it occurs in children.
- Marrow cells have deletion of long arm of chromosome 5 (5q-). In contrast to the 5q- lesion associated with other subtypes of MDS or AML, the break is between q32-q33, whereas in the other cases it is between the commonly deleted region is larger.
- Some patients do not require treatment at the time of diagnosis.
- Significant or symptomatic anemia is improved or ameliorated in the majority of patients treated with lenalidomide, 10 mg/d orally, until improvement occurs or toxicity requires cessation. Improvement ranges from disappearance of signs of the disease, including the 5q- abnormality, to a decrease in transfusion requirements. The maximal response occurs on an average after approximately 5 weeks of treatment.
- Refractory patients with significant anemia may require red cell transfusions. Iron chelation therapy should be instituted based on the three criteria discussed under "Clonal (Refractory) Sideroblastic Anemia: Treatment" above.
- Median survival is approximately 10 years.
- The risk of progression to AML is about 5% to 10% over a prolonged period of observation.

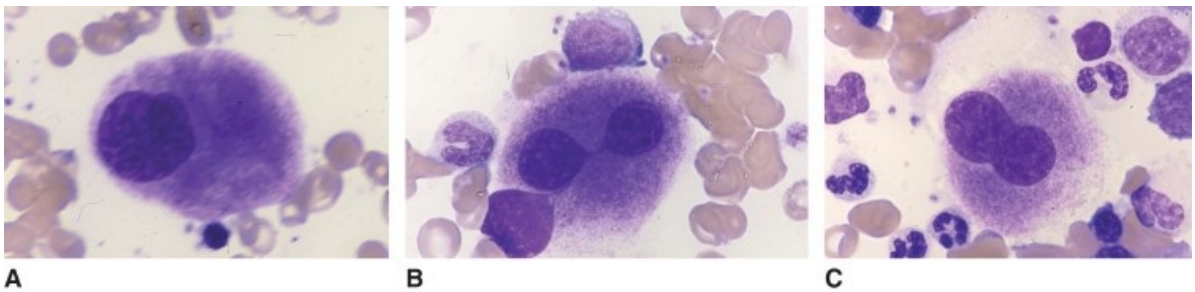


FIGURE 44–3 Composite from marrow films of patient with the 5q–syndrome. Characteristic hypolobulated megakaryocytes. **A.** Monolobed megakaryocyte. **B.** Bilobed megakaryocyte. Lobes connected by a nuclear bridge. **C.** Bilobed megakaryocyte. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

Monosomy 7-Associated Syndromes

- These syndromes are often seen in patients who develop the neoplasm after chemotherapy or high-dose radiotherapy.
- Transformation to AML is common.
- Usually, these conditions are not associated with special clinical features in adults.
- Children often have associated atypical myeloproliferative disease, subacute myelomonocytic leukemia, or juvenile myelomonocytic leukemia that rapidly progresses to AML (see Chap. 46).

OLIGOBLASTIC MYELOGENOUS LEUKEMIAS (REFRACTORY ANEMIA WITH EXCESS BLASTS)

Clinical Features

- Patients have 3% to 19% leukemic blasts in the marrow and 0% to 10% in the blood and may survive for months or years without specific or effective antileukemic therapy.
- These leukemias are also known as smoldering leukemia, pauciblastic leukemia, subacute myelogenous leukemia, or refractory anemia with excess blasts.
- Patients are usually older than 50 years of age, with cytopenias or qualitative cellular abnormalities, as in the clonal cytopenias.
- Thrombocytopenia and/or neutropenia virtually always accompany anemia.
- Evolution into overt polyblastic or AML occurs in approximately 50% of cases.
- Median survival is about 14 to 24 months in different studies, and individual survival in large studies has ranged from 1 to 160 months, highlighting the heterogeneity of disease severity and progression.
- Older age, complex cytogenetics, high blast cell proportions in marrow or blood, and high transfusion requirements are poor prognostic indicators.
- The WHO distinguish patients who have less than or more than 10% blasts in marrow into a type 1 and type 2, respectively.

Treatment

- Therapy should be individualized to the morbidity present, comorbid disorders (eg, physiologic age, diabetes mellitus, heart failure) and the evidence of progression. Some patients may not require treatment initially.
- Periodic patient evaluations are used to detect deteriorating blood counts in a timely manner.
- 5-Azacytidine or decitabine may be used to improve anemia or decrease red cell transfusion requirements. The very good response rate is below 30% of patients treated (see “Clonal (Refractory) Sideroblastic Anemia: Treatment” above for dosing details).
- Therapy with red cell transfusion often is required. If so, iron-chelation therapy should be used if the three criteria (endogenous EPO level, frequency of red cell transfusion needed, and ferritin level) specified in the treatment of “Clonal (Refractory) Sideroblastic Anemia: Treatment,” above, are met (see Chap. 9).
- Platelet transfusion may be required for (1) platelet counts under 5.0×10^9/L, (2) evidence of exaggerated mucocutaneous bleeding, or (3) other significant bleeding episodes.
- ∈-Aminocaproic or tranexamic acid, both antifibrinolytic agents, may be used to decrease the

amount of thrombocytopenic bleeding.

- Thrombopoiesis-stimulating agents (eg, thrombopoietin receptor agonists) are under study for their utility in ameliorating thrombocytopenia in patients with myelodysplasia. Results in a study in low-risk patients have shown effectiveness of romiplostim in half of those treated with platelet counts less than 30×10^9/L.
- Neutropenic fever should be treated promptly with broad-spectrum antibiotics after appropriate cultures for bacteria and fungi are performed.
- If the disease progresses to AML, standard therapy for AML can be considered (see Chap. 45); the remission rate is low and long-term responses are unusual in most patients and especially in patients over age 60 years.
- In patients older than 70 years or who have comorbid conditions or other frailties, attenuated AML therapy can be considered (see Chap. 45).
- In patients not suitable for standard or attenuated standard therapy, a variety of approaches, including low-dose cytarabine, 5-azacytidine or decitabine, etoposide, hydroxyurea, glucocorticoids, singly or in combination, have been tried with limited success.
- The response rate to most therapies is in the range of 5% to 20%. One or another approach accomplishes improvement in different patients, making a standardized approach difficult at this time.
- Allogeneic hematopoietic stem cell transplantation can be curative. The younger the patient, the better the results are with transplantation. The transplant physician involved should make an assessment of all related variables: patient's age, comorbid conditions, prior cytotoxic therapy, probability of a salutary outcome with the disease characteristics in question, patient's level of understanding and interest in the procedure, and others.

RISK CATEGORIES

- Patients with MDS have wide variations in manifestations within a subtype and among subtypes. For example, the number of cytopenias and severity of cytopenias are quite varied among patients.
- Some patients in the same diagnostic subtype do not require treatment, whereas others have highly morbid manifestations, which are life-threatening.
- In order to accommodate this variation among and within subtypes, patients with myelodysplasia have been stratified into prognostic or risk categories using three variables: (1) marrow blast percentage, (2) prognostic category of their cytogenetic findings (good risk, intermediate risk, poor risk), and (3) number of cytopenias (1, 2, or 3; see **Table 44–4**).
- In the aggregate, these prognostic categories predict the median survival, on average, and the likely requirement for early therapy. (See **Tables 44–5** and **44–6**.)
- Details of therapeutic approaches based on prognostic score can be found in *Williams Hematology*, 9th ed, Chap. 87. These approaches use the same therapy described in this chapter under the subtypes of myelodysplasia: red cell transfusion, iron chelation, immunosuppression, DNA-demethylating agents, platelet transfusions, antibiotics, AML therapy, allogeneic hematopoietic stem cell transplantation, and experimental therapies.

TABLE 44–4 **INTERNATIONAL PROGNOSTIC SCORING SYSTEM FOR MYELODYSPLASTIC**

SYNDROMES

Prognostic Variable	Score Value			
	0	0.5	1.0	1.5
Marrow Blast (%)	< 5	5–10	—	11–20
Karyotype	Good	Intermediate	Poor	—
Cytopenias	0,1	2,3	—	—

Risk groups: Low, 0; intermediate-1, 0.5–1.0; intermediate-2, 1.5–2.0; high, ≥2.5. Survival for each risk group is displayed in Table 44–5.

Karyotype: Good score, –Y, del(5q); poor score, complex abnormalities and chromosome 7 abnormalities; intermediate score, all other abnormalities. See "Marrow: Cytogenetics" in text above for further details.

Cytopenias: anemia, hemoglobin < 10 g/dL; neutropenia, absolute neutrophil count < 1.8×10^9/L; thrombocytopenia, platelet count < 100×10^9/L.

Source: *Williams Hematology*, 9th ed, Chap. 87, Table 87–4.

TABLE 44–5 SURVIVAL OF PATIENTS BASED ON THE INTERNATIONAL PROGNOSTIC SCORING SYSTEM

IPSS Group at Diagnosis	No. of Patients	2-Year Survival	5-Year Survival	10-Year Survival	15-Year Survival
Low	267	85%	55%	28%	20%
Intermediate-1	314	70%	35%	17%	12%
Intermediate-2	179	30%	8%	0	–
High	56	5%	0	–	–

IPSS, International Prognostic Scoring System.

Source: *Williams Hematology*, 9th ed, Chap. 87, Table 87–5.

TABLE 44–6 REVISED INTERNATIONAL PROGNOSTIC SCORING SYSTEM FOR MYELODYSPLASTIC SYNDROMES

Cytogenetic Groups	IPSS-R Karyotype Abnormalities
Very good	del(11q), –Y
Good	Normal, del(20q), del(5q) alone or with one other anomaly, del(12p)
Intermediate	+8, del(7q), i(17q), +19, +21, any single or double abnormality not listed, or two or more independent clones
Poor	der(3q), –7, double with del(7q), complex with three abnormalities
Very poor	Complex with more than three abnormalities

IPSS-R Parameter	Categories and Associated Scores				
	Very Good	Good	Intermediate	Poor	Very Poor
Cytogenetic Risk Group	0	1	2	3	4
Marrow blast %	≤2	> 2 – < 5	5–10	> 10	
	0	1	2	3	
Hemoglobin (g/dL)	≥10	8 – < 10	< 8		
	0	1	1.5		
Platelet count ($\times 10^9$/L)	≥100	50 – < 100	< 50		
	0	0.5	1		

Neutrophil count ($\times 10^9$/L)	≥0.8	< 0.8		
	0	0.5		
IPSS-R Risk Group	**Total Score**	**% of Patients**	**Median Survival, Years**	**25% with AML, at Years**
Very low	≤1.5	19	8.8	NR
Low	> 1.5–3	38	5.3	10.8
Intermediate	> 3–4.5	20	3	3.2
High	> 4.5–6	13	1.6	1.4
Very high	> 6	10	0.8	0.73

AML, acute myelogenous leukemia; IPSS-R, International Prognostic Scoring System–Revised; NR, not reached.
These data were found in Greenberg PL et al. Revised international scoring system for myelodysplastic syndromes. Blood 120:2454-65. 2012 and reiterated in this table. Source: *Williams Hematology*, 9th ed, Chap. 87, Table 87–6.

THERAPY-RELATED MYELODYSPLASTIC SYNDROME

- This syndrome usually follows chemotherapy, radiation therapy, or combined modality therapy for solid tumors or lymphomas. Conditioning regimens for autotransplantation in lymphoma and myeloma result in secondary myelodysplasia.
- These cases have a poorer prognosis than de novo cases and are excluded from the calculations of prognostic indices.
- Therapy is very difficult because patients often have another cancer, have gone through periods of intensive cytotoxic therapy, may have other comorbidities, and are often of advanced age.
- Allogeneic hematopoietic stem cell transplantation may be useful in younger patients with a suitable donor and without comorbidities.

TRUE PRELEUKEMIC SYNDROMES

- In the 1950s through the early 1980s, MDS was erroneously described as preleukemia. This descriptive categorization has been discarded. Because these syndromes are clonal, they are neoplastic, and the vital and most critical transition in cell pathology, from polyclonal to monoclonal hematopoiesis, has occurred.
- Many cases have leukemic hematopoiesis in the classic sense (evident leukemic myeloblasts in marrow and blood) and all are leukemic in the pathobiologic sense, because they are all a clonal (neoplastic) disorder originating in a multipotential hematopoietic cell.
- Some polyclonal syndromes are truly preleukemic in the pathobiologic sense, polyclonal disorders that have a propensity to evolve into AML or oligoblastic myelogenous leukemia. Examples are inherited conditions, such as Fanconi anemia, and acquired conditions, such as aplastic anemia. These preleukemia states are listed in Chap. 45, **Table 45–1**.

END NOTE

The term “refractory” is an 80-year-old anachronistic designation, used before these diseases were known to be clonal (neoplastic). Moreover, the response to demethylating agents indicates that many are not “refractory.” The term “excess blasts” is not a pathophysiological entity. These are leukemic blasts qualitatively (genetically) different from normal myeloblasts. Ringed sideroblasts are closely correlated with the mutation *SF3B1,* and the distinction between less than or equal to and more than 15% sideroblasts in prior classifications is not valid; that breakpoint has never had a pathobiological justification for its use. This gene mutation does confer a more favorable prognosis and a decreased propensity of that clone to undergo evolution to AML. Search for this gene mutation should be a part of the diagnosis and classification of clonal anemia. The distinction in blast count of 5 to 9 and 10 to 19 among cases with overt leukemic hematopoiesis is questionable based on a single marrow examination. There are some distinctions in group data, but they are of little usefulness in individual cases in which one must evaluate the degree of abnormality of hematopoiesis globally; determine the patient’s progress over time; and whether and when to use some form of therapy for symptomatic or progressive disease.

Addendum: In May 2016, as *Williams Manual of Hematology*, 9th edition, was being prepared for press, the World Health Organization(WHO) published a modification of the classification of myelodysplastic syndromes as shown in the following Table 44-7.

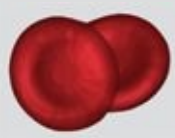
TABLE 44–7 WHO CLASSIFICATION OF MYELODYSPLASTIC SYNDROMES (MDS)

MDS with single lineage dysplasia
MDS with ring sideroblasts (MDS-RS)
MDS-RS and single lineage dysplasia
MDS-RS and multilineage dysplasia
MDS with multilineage dysplasia
MDS with excess blasts
MDS with isolated del(5q)
MDS, unclassifiable
Provisional entity: Refractory cytopenia of childhood

- Important changes include the elimination of the term “refractory” in describing clonal cytopenias such as “refractory anemia”, a 40 year old anachronism.
- The new classification uses “MDS” to initiate each subtype, thereby choosing to retain an anachronism, “dysplasia” to describe neoplasias. (Hypoplasia, hyperplasia, metaplasia, dysplasia, and neoplasia are distinctive pathological entities. Neoplasia is distinguished from dysplasia and other pathological tissue abnormalities cited by being the only one that is monoclonal.)
- MDS is followed by modifiers including: (i) single lineage dysplasia; (ii) multilineage dysplasia; (iii) ring sideroblasts; (iv) isolated del (5q); excess blasts.
- The term “excess blasts” has been retained despite its misleading connotation. Neoplastic (leukemic) blast cells are qualitatively different from normal blast cells and indicate an oligoblastic myelogenous leukemia. The term “excess” is a quantitative distinction, implying hyperplasia not neoplasia, and, thus, pathobiologically erroneous.
- The use of at least 10% dysplastic cells in a lineage to assign it as dysplastic is reaffirmed with important caveats.
- Thresholds are given to define cytopenias: hemoglobin < 10g/dL, neutropenia < 1.0×10^9/L,

thrombocytopenia < 100 × 10^9/L. The physician should recognize that these thresholds are arbitrary and not based on pathophysiology. Patients with MDS may present with abnormally low blood cell counts that have not reached these levels.

- The WHO has recognized that percentage of ring sideroblasts is of no prognostic significance. If the *SF3B1* mutation is uncovered, the diagnosis of MDS-RS(ring sideroblasts) should be made if marrow contains ≥5% ring sideroblasts. In the absence of the *SF3B1* mutation, the diagnosis of MDS-RS(ring sideroblasts) requires ≥15% ring sideroblasts in the marrow.
- The use of the % blast cells to % myeloid cells ratio to correct (increase) the blast percentage in cases with > 50% erythroblasts in marrow has been revisited and has resulted in a change of the diagnosis of acute erythroid leukemia in such cases to MDS unless the blasts represent ≥20% of all marrow cells or the erythroid cells are > 80% and 30% are proerythroblasts.
- The reader is referred to Arber D, Orazi A, Hasserjian R, et al. The 2016 revision of the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 127:2391-2405, 2016 for further details.



For a more detailed discussion, see Rafael Bejar and David Steensma: Myelodysplastic Syndromes, Chap. 87 in *Williams Hematology*, 9th ed.

CHAPTER 45
The Acute Myelogenous Leukemias

- Acute myelogenous leukemia (AML) is a malignancy originating in the lymphohematopoietic stem cell or a closely related multipotential hematopoietic cell.
- It is characterized by clonal proliferation of abnormal blast cells in the marrow and impaired production of normal blood cells, resulting in anemia; thrombocytopenia; and low, normal, or high white cell counts depending on the concentration of leukemic cells in the blood.
- AML occurs in nine morphologic (phenotypic) variants, each with characteristic cytologic, genetic, and sometimes clinical features.
- It has been found to have innumerable (more than 150) mutated genetic driver and cooperating oncogenes.

ETIOLOGY AND PATHOGENESIS

- The chronic clonal myeloid diseases may undergo clonal evolution to AML (eg, polycythemia vera, Chap. 41; essential thrombocythemia, Chap. 42; myelofibrosis, Chap. 47; myelodysplastic syndromes, Chap. 44; the chronic myelogenous leukemias, Chap. 46).
- AML develops with increased frequency in patients with certain congenital (Down syndrome) or inherited abnormalities (eg, Fanconi anemia, familial platelet syndrome) as shown in **Table 45–1**.
- Nonsyndromic, familial occurrence, indicating an inherited predisposition gene, has been documented but is very uncommon.
- Most cases arise de novo and are associated with either acquired cytogenetic changes, including translocation, inversions, deletions, and other forms of aneuploidy or pseudodiploidy. These changes lead to the mutation of proto-oncogenes and the formation of oncogenes. Frequently, the latter encode mutant transcription factors resulting in disruption of cell signaling pathways that cause malignant transformation.
- In the absence of chromosome abnormalities, one can identify specific gene mutations that account for the disruption in normal hematopoiesis and the establishment of a leukemic clone.
- Thus, AML results from a series of somatic mutations in a multipotential hematopoietic cell or, in a small proportion of cases, a more differentiated, lineage restricted progenitor cell. In acute promyelocytic leukemia (APL), some cases of monocytic leukemia, and some young persons with other forms of AML, the disease may originate in a mutated granulocytic-monocytic progenitor cell that is transformed into a leukemic stem cell.
- The mutations resulting in AML disrupt stem cell differentiation and unilineage progenitor maturation, regulation of proliferation, and of cell survival (apoptosis) in varying combinations. This complexity results in many phenotypes.

TABLE 45–1 CONDITIONS PREDISPOSING TO DEVELOPMENT OF ACUTE MYELOGENOUS LEUKEMIA

- Environmental (external) factors
  - Alkylating agents, topoisomerase II inhibitors, and other cytotoxic drugs
  - Radiation
  - Tobacco smoke
  - Benzene
- Acquired diseases
  - Clonal myeloid diseases
    - Chronic myelogenous leukemia
    - Essential thrombocythemia
    - Myelodysplastic syndromes
    - Paroxysmal nocturnal hemoglobinuria
    - Polycythemia vera
    - Primary myelofibrosis
  - Other hematopoietic disorders
    - Aplastic anemia
    - Eosinophilic fasciitis
    - Myeloma
  - Other disorders
    - Human immunodeficiency virus infection
    - Langerhans cell histiocytosis
    - Polyendocrine disorders
    - Thyroid disorders
- Inherited or congenital conditions
    - Sibling with AML
    - Amegakaryocytic thrombocytopenia, congenital
    - Ataxia-pancytopenia
    - Bloom syndrome
    - Congenital agranulocytosis (Kostmann syndrome)
    - Chronic thrombocytopenia with chromosome 21q 22.12 microdeletion
    - Diamond-Blackfan syndrome
    - Down syndrome
    - Dubowitz syndrome
    - Dyskeratosis congenita
    - Familial (pure, nonsyndromic) AML
    - Familial AML with *CEBPA* mutations
    - Familial platelet disorder
    - Fanconi anemia
    - MonoMAC and Emberger syndromes (*GATA2* mutations)
    - Naxos syndrome
    - Neurofibromatosis 1
    - Noonan syndrome
    - Poland syndrome
    - Rothmund-Thomson syndrome
    - Seckel syndrome
    - Shwachman syndrome
    - Werner syndrome (progeria)
    - Wolf-Hirschhorn syndrome
    - WT syndrome

Source: *Williams Hematology*, 9th ed, Chap. 88, Table 88–1.

EPIDEMIOLOGY

- AML accounts for 80% of acute leukemias in adults and 15% to 20% in children.

- AML is the most frequent leukemia in neonates. This results in a bimodal incidence curve with a peak at less than 1 year of age of approximately 2 per 100,000 infants, dropping to approximately 0.4 cases per 100,000 at age 7 years, and then increasing to 1.0 per 100,000 by age 25 years. Thereafter, incidence increases exponentially to 20 cases per 100,000 persons in octogenarians (**Figure 45–1**).
- An exception to the striking change in incidence with age in adults is found in APL in which the incidence by age does not vary as significantly.
- Four exposures have been established as causative factors. These include high-dose radiation; higher-dose chronic benzene exposure, usually in an industrial setting; treatment of other cancers or severe autoimmune syndromes with alkylating agents, topoisomerase II inhibitors, or some other cytotoxic drugs; and prolonged tobacco smoking. Numerous other possible environmental factors have been studied but are unproven as causal factors.
- The risk of AML in a nonidentical sibling is approximately 2.5-fold that of unrelated individuals under age 15 years in persons of European descent.
- The risk of AML appears to be increased in Jews of Eastern European descent and the APL subtype increased among Latinos.

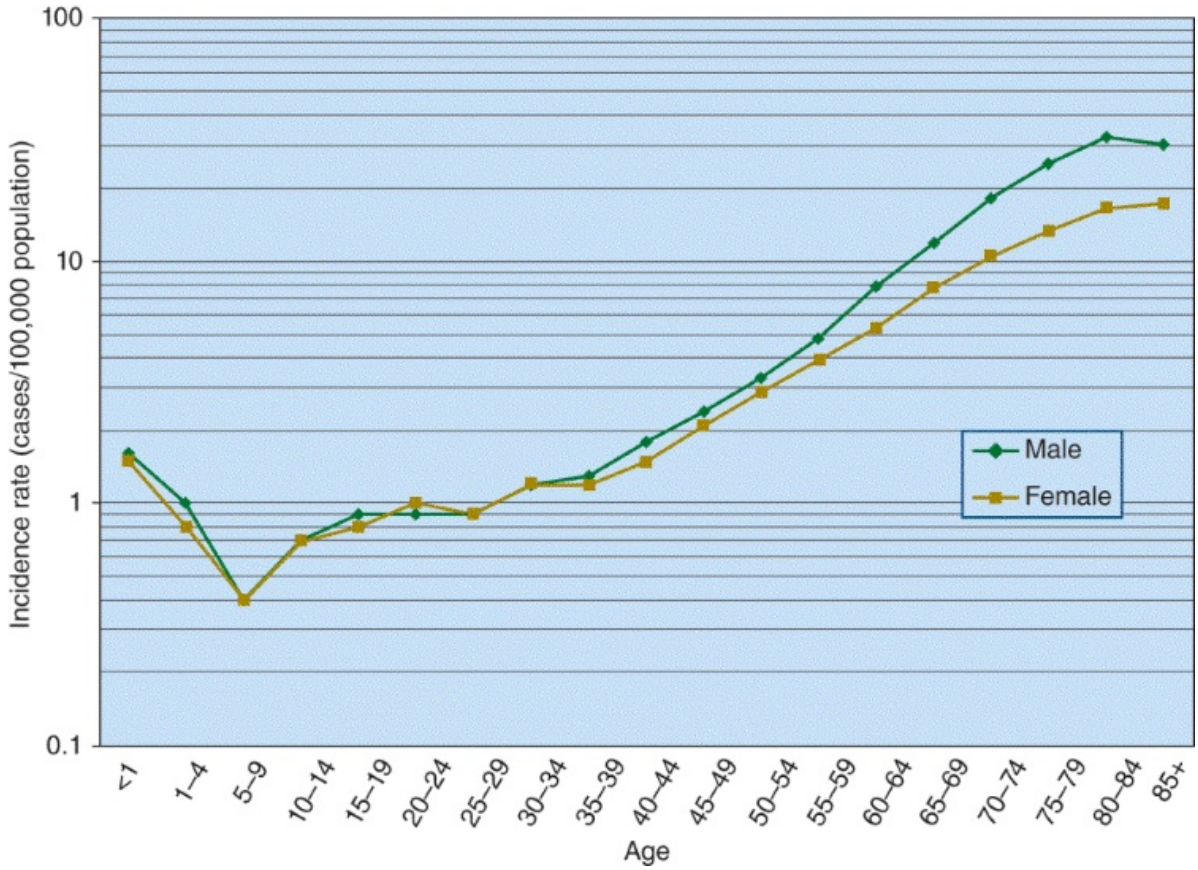


FIGURE 45–1 The annual incidence of acute myelogenous leukemia (AML) as a function of age. There is a relatively small increase to approximately 1.5 cases per 100,000 persons in the first year of life, representing congenital, neonatal, and infant AML. The incidence falls to a nadir of 0.4 new cases per 100,000 persons over the first 10 years of life and then rises again to 1 case per 100,000 in the second decade of life. From approximately 20 to 25 years of age, the incidence increases exponentially (log-linear) to approximately 25 cases per 100,000 population in octogenarians. (Source: *Williams Hematology*, 9th ed, Chap 88, Fig. 88–1.)

CLASSIFICATION

- AML develops clinically in nine morphologic variants that can be identified by a combination of blood cell and marrow morphology on stained slides, and immunophenotype (cluster of differentiation or CD profile) measured by flow cytometry (**Table 45–2**) and histochemical analysis, if necessary. Cytogenetic and gene mutational analysis provides a second level of classification. The diversity of specific cytogenetic abnormalities (hundreds) makes this useful only for the few most prevalent chromosome alterations (see "Laboratory Features" section, below).

TABLE 45–2 IMMUNOLOGIC PHENOTYPES OF ACUTE MYELOGENOUS LEUKEMIA

Phenotype	Usually Positive
Myeloblastic	CD11b, CD13, CD15, CD33, CD117, HLA-DR
Myelomonocytic	CD11b, CD13, CD14, CD15, CD32, CD33, HLA-DR
Erythroid	Glycophorin, spectrin, ABH antigens, carbonic anhydrase I, HLA-DR, CD71 (transferrin receptor)
Promyelocytic	CD13, CD33
Monocytic	CD11b, 11c, CD13, CD14, CD33, CD65, HLA-DR
Megakaryoblastic	CD34, CD41, CD42, CD61, anti-von Willebrand factor
Basophilic	CD11b, CD13, CD33, CD123, CD203c
Mast cell	CD13, CD33, CD117
Dendritic cell	CD2AFCD4CD56CD123CD303

Source: *Williams Hematology*, 9th ed, Chap. 88, Table 88–2.

CLINICAL FEATURES

- Frequent presenting symptoms and signs are those reflecting anemia: pallor, fatigue, weakness, palpitations, and dyspnea on exertion; or thrombocytopenia: ecchymoses, petechiae, epistaxis, gingival bleeding, conjunctival hemorrhages, and prolonged bleeding after minor cuts.
- Minor pyogenic infections of the skin are common. Major infections are uncommon at diagnosis, prior to cytotoxic therapy.
- Anorexia and weight loss may occur.
- Low-grade fever may be present at onset.
- Mild splenomegaly or hepatomegaly is present in about one-third of patients. Lymph node enlargement is uncommon, except with the monocytic variant.
- Leukemic cells may infiltrate any organ in the body, but consequent organ dysfunction is unusual.
- Occasionally, large accumulations of myeloblasts (myeloid sarcoma) may develop in virtually any tissue.
- Leukemic monoblasts and promonocytes frequently infiltrate tissues, and these sites can be symptomatic (eg, leukemia cutis, gingival hyperplasia, lymphadenopathy, and others).

LABORATORY FEATURES

- Anemia and thrombocytopenia are nearly always present at diagnosis. Half the patients with AML have a platelet count less than 50×10^9/L.
- Red cell morphology is mildly abnormal (anisocytosis and occasional misshapen cells), although in occasional cases, more marked dysmorphia occurs.
- Total leukocyte count is below 5.0×10^9/L in about one-half of patients, and the absolute neutrophil count is less than 1.0×10^9/L in more than one-half of patients at diagnosis. Mature neutrophils may be hypersegmented, hyposegmented, or hypogranular.
- Myeloblasts comprise from 3% to 95% of the leukocytes in the blood, and a small percent of the blast cells may contain Auer rods.
- Marrow contains leukemic blast cells, identified as myelogenous by reactivity with cytochemical stains (eg, peroxidase), presence of Auer rods, or reactivity with antibodies to epitopes specific for myeloblasts or derivative cells (**Table 45–2**).
- The World Health Organization defines AML as having greater than or equal to 20% blasts in the marrow. This breakpoint is not based on rational biologic considerations. APL, acute monocytic leukemia, acute myelomonocytic leukemia, and AML with evidence of maturation may not and often do not have greater than or equal to 20% blasts in the marrow (see also Chap. 40). Moreover, patients with 10% to 20% blasts in the marrow may behave as AML or progress rapidly to such behavior and require treatment for AML. The physician should eschew medicine by algorithm. Some of these approaches may be required for multi-institutional clinical trials but should not be transposed to the care of individual patients.
- Overt cytogenetic abnormalities (aneuploidy or pseudodiploidy) are present in about half to three-fourths of patients. Abnormalities such as t(8;21), t(15;17), inv 16, and translocations involving 11q are the most common, but several hundred unique cytogenetic abnormalities have been described in the cells of AML patients. The most prevalent abnormalities are shown in **Table 45–3**.
- The frequency of cytogenetic abnormalities with a favorable progress is age-dependent; they are more common in younger patients (**Table 45–4**).
- The most prevalent genetic mutations found in patients with AML without a cytogenetic abnormality are shown in **Table 45–5**.
- Serum uric acid and lactic acid dehydrogenase levels are frequently elevated.
- Electrolyte abnormalities are infrequent, but severe hypokalemia may occur, and spurious hyperkalemia may be found in patients with very high leukocyte counts.
- Patients with very high leukocyte counts may also have spurious hypoglycemia and hypoxia as a result of consumption of glucose or oxygen by the blast cells after the specimen is obtained.
- Hypercalcemia and hypophosphatemia may be present.

TABLE 45–3 CLINICAL CORRELATES OF FREQUENT CYTOGENETIC ABNORMALITIES OBSERVED IN ACUTE MYELOGENOUS LEUKEMIA (AML)

Chromosome Abnormality	Genes Affected	Clinical Correlation
Loss or gain of chromosome		
Deletions of part or all of chromosome 5 or 7	Not defined	Frequent in patients with AML occurring de novo and in patients with history of chemical, drug, or radiation exposure and/or previous hematologic disease.
Trisomy 8	Not defined	Very common abnormality in acute myeloblastic leukemia. Poor

		prognosis, often a secondary change.
Translocation		
t(8;21) (q22;q22)	*RUNX1(AML1)–RUNX1T1(ETO)*	Present in ~8% of patients < 50 years old and in 3% of patients > 50 years old with AML. Approximately 75% of cases have additional cytogenetic abnormalities, including loss of Y in males or X in females. Secondary cooperative mutations of *KRAS, NRAS, KIT* common. Present in ~40% of myelomonocytic phenotype. Higher frequency of myeloid sarcomas.
t(15;17) (q31; q22)	*PML-RAR-α*	Represents ~6% of cases of AML. Translocation involving chromosome 17, t(15;17), t(11;17), or t(5;17) is present in most cases of promyelocytic leukemia.
t(9;11); (p22; q23)	*MLL (especially MLLT3)*	Present in ~7% of cases of AML. Associated with monocytic leukemia. 11q23 translocations in 60% of infants with AML and carries poor prognosis. Rearranges *MLL* gene. Many translocation partners for 11q23 translocation. *MLL1, MLL4, MLL10* may also result in AML phenotype.
t(9;22) (q34; q22)	*BCR-ABL1*	Present in ~2% of patients with AML.
t(1;22) (p13; q13)	*RBMIS-MKL1*	< 1% of cases of AML. Admixture of myeloblasts, megakaryoblasts, micromegakaryocytes with cytoplasmic blebbing, dysmorphic megakaryocytes. Reticulin fibrosis common.
t(10;11) (p12-13; q14-21)	*PICALM-MLLT10*	Outcome similar to that of intermediate prognosis group; more extramedullary disease and CD7 expression.
Inversion		
Inv(16) (p13.1; q22) or t(16;16) (p13.1; q22)	*CBF-β MYH11*	Present in ~8% of patients < 50 years of age and in ~3% of patients > 50 years of age with AML; often acute myelomonocytic phenotype; associated with increased marrow eosinophils; predisposition to cervical lymphadenopathy, better response to therapy. Predisposed to myeloid sarcoma.
Inv(3) (q21q26.2)	*RPN1-EVI1*	~1% of cases of AML. Approximately 85% of cases with normal or increased platelet count. Marrow has increased dysmorphic, hypolobulated megakaryocytes. Hepatosplenomegaly more frequent than usual in AML.

Source: *Williams Hematology*, 9th ed, Chap. 88, Table 88–4.

TABLE 45–4 FREQUENCY OF CYTOGENETIC FINDINGS WITH A MORE FAVORABLE PROGNOSIS BY AGE GROUP

Age (Years)	No. of Cases Studied	t(8;21) (No. of Cases)	t(15;17) (No. of Cases)	Inv16/t(16;16) (No. of Cases)	Total (No. of Cases)	Favorable Karyotypes (% of all Cases)
10–39	307	27	38	33	98	32
40–59	584	36	28	28	92	16
60–69	579	18	24	21	63	11
70–79	381	5	7	5	17	4.5
> 80	45	1	2	0	3	6.6
Total	1896	87	99	87	273	22

These observations were made in Germany by Claudia Schoch and colleagues and kindly provided to the authors.

Source: *Williams Hematology*, 9th ed, Chap. 88, Table 88–9.

TABLE 45–5 COMMONLY MUTATED CYTOGENETICALLY NORMAL ACUTE MYELOGENOUS LEUKEMIA (AML)

Mutated Gene	Approximate Frequency in AML with Normal Karyotype (%)	Implication	Comments
NPM1	50	More favorable outcomes	Most frequently mutated gene in AML. Allogenic transplantation not needed in first remission if this mutation occurs in absence of mutated *FLT3*-ITD.
FLT3 ITD	40	Less favorable outcomes	
DNMT3A	20	Less favorable outcomes	Seen more often in AML patients with normal cytogenetics. Mutant *NPM1*, *FLT3-ITD*, and *IDH1* have been found more frequently in AML patients with *DNMT3A* mutations compared to those with wild-type *DNMT3A*.
RUNX1	15	Less favorable outcomes	
TET2	15	Less favorable outcomes	Coincidence of mutated *TET2 with NPM1* mutation in the absence of *FLT3-ITD* mutation predicts a less favorable outcome.
CEPBA	15	More favorable outcomes	Only cases with double mutations associated with favorable outcomes.
NRAS	10	Little effect on prognosis	
IDH1 or IDH2	10	Little effect on outcomes	More frequent in AML patients with normal cytogenetics. Frequently associated with *NPM1*. Adverse prognostic factor if present with mutated *NPM1* without *FLT3-ITD*. Serum 2-hydroxyglutarate levels indicate high probability of *IDH* mutation.
MLL-PTD	8	Less favorable outcomes	
WT1	6	Less favorable outcomes	More frequent in females than in males (6.6 vs 4.7%; $P = .014$) and in patients < 60 than in patients > 60 years ($P < .001$).
FLT3-TKD	6	Little effect on outcomes	May appear after use of FLT3-ITD inhibitor.

AML, acute myelogenous leukemia; *CEPBα*, CCAAT/enhancer binding protein alpha; *DNMT3A*, DNA methyltransferase 3A; *FLT*, FMS-like tyrosine kinase; *IDH*, isocitrate dehydrogenase; ITD, internal tandem duplication; *MLL*, myeloid-lymphoid (mixed-lineage) leukemia; *NPM*, nucleophosmin; PTD, partial tandem deletion; *RAS*, rat sarcoma; *RUNX*, Runt-related transcription factor; TET, ten-eleven translocation; TKD, tyrosine kinase domain; *WT*, Wilms tumor.

Gene frequencies are approximations, with some variation from study to study. Outcome statement does not reflect effect of interacting mutations unless otherwise noted in comments. Outcome statements are based on consensus and vary from one study to another.

Source: *Williams Hematology*, 9th ed, Chap. 88, Table 88–2.

MARROW NECROSIS

- Twenty-five percent of cases occur as a complication of AML.
- Bone pain (80% of cases) and fever (70% of cases) are the two most frequent signs.
- Marrow aspirate is watery and serosanguineous. Marrow cells are indistinct and lose staining characteristics with pyknotic cells exhibiting karyorrhexis.
- Prognosis is most closely related to underlying disease.

HYPERLEUKOCYTOSIS

- Signs and symptoms are due to extreme elevations of the leukocyte count, usually to greater than 100×10^9/L, appear in about 5% of patients.
- Leukostasis is most likely to occur (1) in the circulation of the central nervous system, leading to intracerebral hemorrhage; (2) in the lungs, resulting in pulmonary insufficiency; or (3) in the penis, causing priapism (see Chap. 40).

ATYPICAL PRESENTATIONS OF AML

- *Hypoplastic leukemia.* AML may present with pancytopenia and a hypoplastic marrow. Careful microscopic and cell flow analysis examinations usually identify leukemic blast cells in marrow.
- *Oligoblastic myelogenous leukemia.* The disease may present with anemia and thrombocytopenia and a lower proportion of blast cells in the blood and marrow. This presentation is often referred to as myelodysplasia (specifically, refractory anemia with excess blasts) and is discussed in Chap. 44. Although median survival without remission-induction therapy is measured at about 20 months, it may be morbidly symptomatic, be progressive soon after diagnosis, and in the appropriate patient require AML-type therapy, especially in cases in which the marrow blast percentage is 10% to 20% at the time of diagnosis.
- *Mediastinal germ-cell tumors* and *AML* may coexist, and there is evidence for a clonal identity of the neoplastic cells of the two tumors.

AML IN NEONATES

- *Transient abnormal myeloproliferation* (ie, markedly elevated leukocyte count with blast cells in the blood and marrow) is present at birth or appears shortly thereafter and resolves slowly over weeks or months without treatment.
- Similar cases with a cytogenetic abnormality may resolve and then later reappear as acute leukemia. Such disorders have been referred to as *transient leukemia.* These events occur in approximately 10% of newborns with Down syndrome.
- Apparently phenotypically normal newborns may have *congenital leukemia* or develop *neonatal leukemia.* However, these syndromes are 10 times more common in infants with Down syndrome. The leukemia in Down syndrome is usually acute megakaryocytic leukemia and is very responsive to chemotherapy.

AML IN OLDER PATIENTS

- Higher frequency of unfavorable prognostic cytogenetic changes
- Higher frequency of drug-resistant phenotypes
- Higher frequency of comorbid conditions
- Lower tolerance to intensive chemotherapy
- Lower rate of remission and shorter survival with current therapeutic approaches

HYBRID (BIPHENOTYPIC) LEUKEMIAS

- These are leukemias in which individual cells may have both myeloid and lymphoid markers (chimeric), or in which individual cells may have either myeloid or lymphoid markers but appear to arise from the same clone (mosaic). In some cases, individual cells may have markers for two or more myeloid lineages, such as granulocytic and megakaryocytic.
- Myeloid–natural killer cell and t(8;13) myeloid–lymphoid hybrids are two explicit syndromes representing this phenomenon.
- *Mixed leukemias* are rare entities in which myeloid and lymphoid cells are present simultaneously, each derived from a separate clone (eg, chronic myelogenous leukemia and chronic lymphocytic leukemia occurring simultaneously).

MORPHOLOGIC VARIANTS OF AML

- **Table 45–6** presents the features of the morphologic variants of AML.
- The most common variants have phenotypic features of granulocytic, monocytic, erythroid, or megakaryocytic cells.
- Acute eosinophilic, basophilic, mastocytic, or dendritic leukemias arising de novo are rare forms of AML.
- Images of morphologic variants and features of leukemic cells are shown in **Figure 45–2**.

TABLE 45–6 MORPHOLOGIC VARIANTS OF ACUTE MYELOGENOUS ANEMIA

Variant	Cytologic Features	Special Clinical Features	Special Laboratory Features
Acute myeloblastic leukemia (M0, M1, M2)	1. Myeloblasts range from 20%–90% of marrow cells. Cytoplasm occasionally contains Auer bodies. Nucleus shows fine reticular pattern and distinct nucleolus (1 or 2 usually). 2. Blast cells are sudanophilic. They are positive for myeloperoxidase and chloroacetate esterase, negative for nonspecific esterase, and negative or diffusely positive for PAS (no clumps or blocks). 3. Electron microscopy shows cytoplasmic primary granules.	1. Most common in adults, and most frequent variety in infants. 2. Three morphologic-cytochemical types (M0, M1, M2)	1. Chromosomes +8, –5, –7, del(11q) and complex abnormalities common. *RUNX1(AML1)* and *FLT3* mutations occur in approximately 20%–25% of cases. 2. M0 type blast cells positive with antibody to myeloperoxidase and anti-CD34 and CD13 or CD33 coexpression. *AML1* mutations in ~25%. 3. M1 expresses CD13 and CD33. Positive for myeloperoxidase by cytochemistry. 4. M2 AML with maturation often

			associated with t(8;21) karyotype. 5. M2 AML with t(6;9)(p23; q34), an uncommon variant, is associated with marrow basophilia, a high blast count, a high frequency of *FLT3*-ITD, and a poor outcome.
Acute promyelocytic leukemia (M3, M3v)	1. Leukemic cells resemble promyelocytes. They have large atypical primary granules and a kidney-shaped nucleus. Branched or adherent Auer rods are common. 2. Peroxidase stain intensely positive. 3. A variant has microgranules (M3v), otherwise the same course and prognosis.	1. Usually in adults. 2. Hypofibrinogenemia and hemorrhage common. 3. Leukemic cells mature in response to all-*trans*-retinoic acid.	1. Cell contains t(15;17) or other translocation involving chromosome 17 (*RAR*-α gene). 2. Cells are HLA-DR negative.
Acute myelomonocytic leukemia (M4, M4Eo)	1. Both myeloblastic and monoblastic leukemic cells in blood and marrow. 2. Peroxidase-, Sudan-, chloroacetate esterase-, and nonspecific esterase-positive cells. 3. M4Eo variant has marrow eosinophilia.	1. Similar to myeloblastic leukemia but with more frequent extramedullary disease. 2. Mildly elevated serum and urine lysozyme.	1. Leukemic cells in eosinophilic variant (M4Eo) usually have inversion or translocation of chromosome 16.
Acute monocytic leukemia (M5)	1. Leukemia cells are large; nuclear cytoplasmic ratio lower than myeloblast. Cytoplasm contains fine granules. Auer rods are rare. Nucleus is convoluted and cell simulates promonocytes (M5a) or may simulate monoblasts (M5b) and contain large nucleoli. 2. Nonspecific esterase-positive inhibited by NaF; Sudan-, peroxidase-, and chloroacetate esterase-negative. PAS occurs in granules, blocks.	1. Seen in children or young adults. 2. Gum, CNS, lymph node, and extramedullary infiltrations are common. 3. DIC occurs. 4. Plasma and urine lysozyme elevated. 5. Hyperleukocytosis common.	1. t(4;11) common in infants. 2. Rearrangement of q11;q23 very frequent.
Acute erythroid leukemia (M6)	1. Abnormal erythroblasts are in abundance initially in marrow and often in blood. Later the morphologic findings may be indistinguishable from those of AML.	1. Pancytopenia common at diagnosis.	1. Cells reactive with antihemoglobin antibody. Erythroblasts usually are strongly PAS and CD71-positive, express ABH blood group antigens. 2. Cells reactive with anti–Rc-84 (antihuman erythroleukemia cell-line antigen).
Acute megakaryocytic leukemia (M7)	1. Small blasts with pale agranular cytoplasm and cytoplasmic blebs. May mimic lymphoblasts of medium to larger size. 2. Leukemic cells with megakaryocytic morphology may coexist with megakaryoblasts.	1. Usually presents with pancytopenia. 2. Markedly elevated serum lactic acid dehydrogenase levels. 3. Marrow aspirates are usually “dry taps” because of the invariable presence of	1. Antigens of von Willebrand factor, and glycoprotein Ib (CD42), IIb/IIIa (CD41), IIIa (CD61) on blast cells. 2. Platelet peroxidase positive.

		myelofibrosis. 4. Common phenotype in the AML of Down syndrome.	
Acute eosinophilic leukemia	1. Mixture of blasts and cells with dysmorphic eosinophilic granules (smaller and less refractile).	1. Hepatomegaly, splenomegaly, lymphadenopathy may be prominent. 2. Absence of neurologic, respiratory, or cardiac signs or symptoms characteristic of chronic eosinophilic leukemia (clonal hypereosinophilic syndrome).	1. Cyanide-resistant peroxidase stains eosinophilic granules. TEM shows eosinophilic granules to be smaller and missing central crystalloid. 2. Biopsy may show Charcot-Leyden crystals in skin, marrow, or other sites of eosinophil accumulation.
Acute basophilic leukemia	1. Mixture of blast cells and cells with basophilic granules in blood and marrow.	1. Often has hepatomegaly and or splenomegaly; symptoms often present. 2. Rash with urticaria, headaches, prominent gastrointestinal symptoms.	1. CD9 -, CD11b-, CD25-, CD123-positive cells are usually present. 2. Toluidine blue-positive cells. 3. Hyperhistaminemia and hyperhistaminuria. 4. Cells negative for tryptase but positive for histidine decarboxylase.
Acute mast cell leukemia	1. Mast cells in blood and marrow. Most contain granules but some are agranular and may simulate monocytes.	1. Fever, headache, flushing of face and trunk, pruritus may be present. 2. Abdominal pain, peptic ulcer, bone pain, diarrhea more common than other AML subtypes. 3. Hepatomegaly, splenomegaly common. 4. Hemorrhagic diathesis may be evident.	1. CD13, CD33, CD68, CD117 often positive. 2. Cells positive for tryptase staining and serum tryptase elevated. 3. Hyperhistaminemia and hyperhistaminuria.
Dendritic cell	1. Blast cells with irregular nuclei. Microvacuoles, pseudopodia may be evident.	1. Cutaneous involvement universal. 2. Often starts in skin and involves marrow later. 3. May have myelodysplastic cell features.	1. CD4, CD43, CD56, CD68, CD123, CD303 2. T-cell and B-cell genes germline. 3. Median survival 12 months. AML therapy followed by stem cell transplant best approach in younger patients.

AML, acute myelogenous leukemia; DIC, disseminated intravascular coagulation; HLA-DR, human leukocyte antigen-D related; NaF, sodium fluoride; PAS, periodic acid–Schiff; RAR, retinoic acid receptor; TEM, transmission electron microscopy.
Note: Parentheses indicate French-American-British (FAB) designation M0 through M7.
Source: *Williams Hematology*, 9th ed, Chap. 88, Table 88–5.

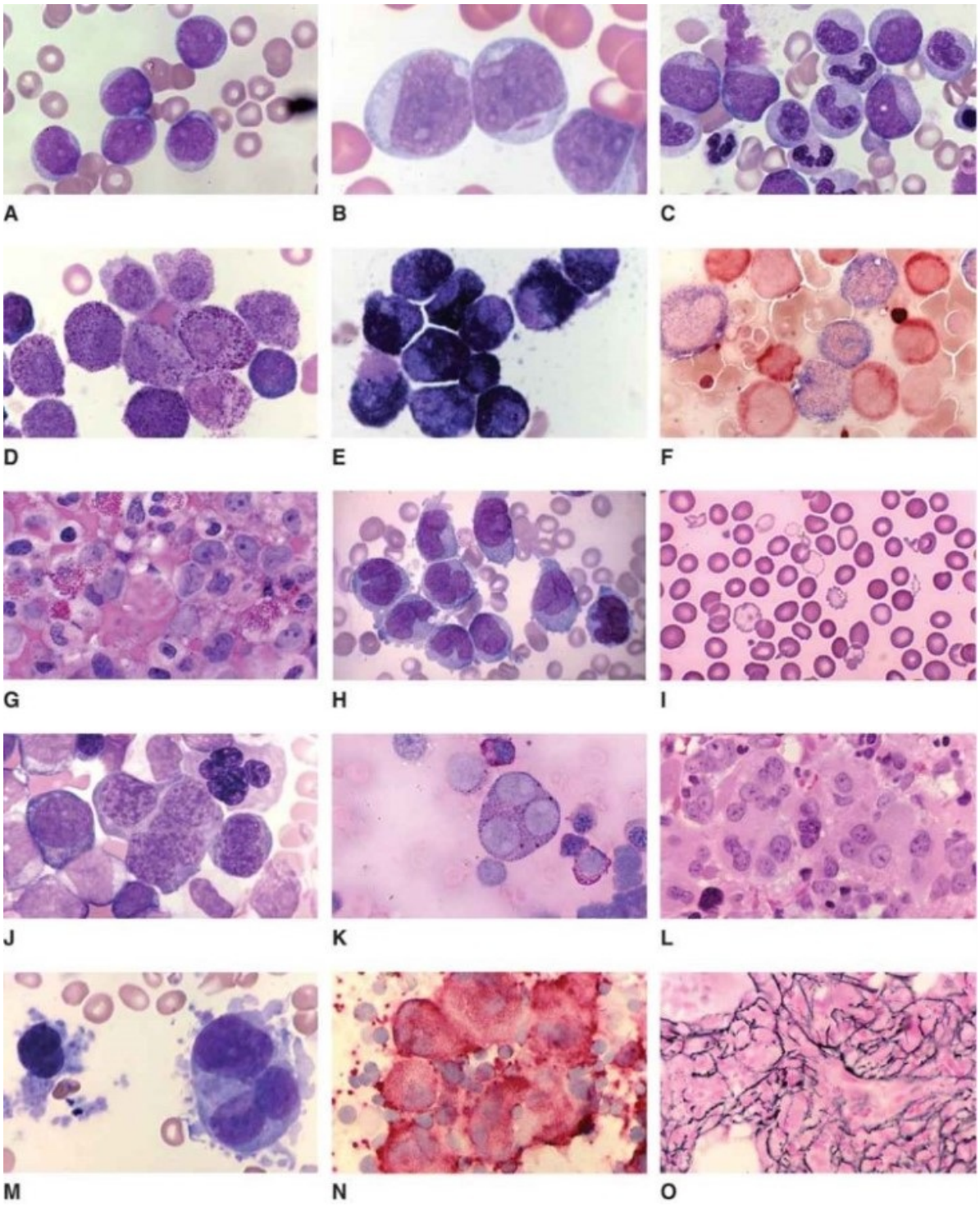


FIGURE 45–2 Blood and marrow images of major subtypes of acute myelogenous leukemia (AML). **A.** Blood film of AML without maturation (acute myeloblastic leukemia). Five myeloblasts are evident. High nuclear-to-cytoplasmic ratio. Agranular cells. Nucleoli in each cell. **B.** Blood film. AML without maturation (acute myeloblastic leukemia). Three myeloblasts, one containing an Auer rod. **C.** Marrow film. AML with maturation. Three leukemic myeloblasts admixed with myelocytes, bands, and segmented neutrophils. **D.** Blood film. Acute promyelocytic leukemia. Majority of cells are heavily granulated leukemic promyelocytes. **E.** Blood film. Acute promyelocytic leukemia. Myeloperoxidase stain. Intensely positive. Numerous stained (black) granules in cytoplasm of leukemic progranulocytes. **F.** Blood film. Acute myelomonocytic leukemia. Double esterase stain. Leukemic monocytic cells stained dark blue and leukemic neutrophil precursors stained reddish-brown. **G.** Marrow film. AML with inv16. Note high proportion of eosinophils in field. Note myeloblasts with very large nucleoli at upper right. Also, intermediate leukemic granulocytic forms. **H.** Blood film. Acute monocytic leukemia. Leukemic cells have characteristics of monocytes with agranular gray cytoplasm and reniform or folded nuclei with characteristic chromatin staining. This case had hyperleukocytosis as evident by leukemic monocyte frequency in blood film. **I.** Blood film. Acute erythroid leukemia. Note population of extremely hypochromic cells with scattered bizarre-shaped poikilocytes admixed with normal-appearing red cells. **J.** Marrow film. Acute erythroid leukemia. Giant erythroblasts with multilobulated nuclei. **K.** Marrow film. Acute erythroid leukemia. Note giant trinucleate erythroblast and other leukemic erythroblasts with periodic acid-Schiff–positive cytoplasmic staining (reddish granules). **L.** Marrow

section. Acute megakaryoblastic leukemia. Marrow replaced with atypical two- and three-lobed leukemic megakaryocytes with bold nucleoli. **M.** Marrow film. Acute megakaryoblastic leukemia. Marrow replaced with atypical megakaryocytes and megakaryoblasts with cytoplasmic disorganization, fragmentation, and budding. **N.** Marrow film. Acute megakaryoblastic leukemia. Marrow replaced with atypical megakaryocytes and megakaryoblasts staining for platelet glycoprotein IIIA (reddish-brown). Platelets in background also stained. **O.** Marrow section. Acute megakaryoblastic leukemia. Argentophilic (silver) stain shows marked increase in collagen, type III fibrils (marrow reticulin fibrosis), characteristic of this AML subtype. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

DIFFERENTIAL DIAGNOSIS

- Extensive proliferation of promyelocytes in the marrow may occur on recovery from agranulocytosis induced by drugs or bacterial infection and transiently may mimic APL. This blood and marrow appearance resolves spontaneously in several days and has been called *pseudoleukemia*.
- In patients with hypoplastic marrows, it may be difficult to differentiate hypoplastic acute leukemia from aplastic anemia. Careful, and sometimes repeated, evaluation of blood and marrow cytology should permit the correct diagnosis.
- Leukemoid reactions and nonleukemic pancytopenias do not have an increase in leukemic myeloblasts in the marrow or blood.

THERAPY, COURSE, AND PROGNOSIS

- The patient and the family should be informed about the nature of the disease, the treatment, and the potential side effects of the treatment.
- Treatment should be initiated as soon as possible after diagnosis, unless the patient is so frail or burdened by unrelated illness to make treatment inadvisable based on mutual understanding.
- Associated problems such as hemorrhage, infection, or anemia should be treated concurrently.
- Pretreatment laboratory studies should establish the specific diagnosis using immunologic, cytogenetic, and molecular genetic techniques, and assess the general condition of the patient, including blood chemistry tests, radiographic examinations, and cardiac studies, as indicated. Hemostasis should also be evaluated, in detail if screening tests show any abnormalities, if severe thrombocytopenia is present, or if the patient has APL or monocytic leukemia.
- The suspicion of APL based on blood or marrow morphology should result in immediate treatment with all-trans retinoic acid, even in the emergency department before admission, in an effort to reduce the incidence of intracranial hemorrhage (see APL Treatment section below and **Table 45-8**).
- An indwelling central venous catheter should be inserted prior to beginning treatment.
- Treatment with allopurinol, usually 300 mg daily orally, should be given if the serum uric acid level is greater than 7 mg/dL, if the marrow is hypercellular with increased blast cells, or if the blood blast cell count is moderately or markedly elevated. Intravenous rasburicase, a recombinant form of uric oxidase, a rapidly acting preparation, is also available for more urgent situations. Drugs to lower uric acid should be discontinued after control of the uric acid level and cytoreduction.
- Exposure to pathogenic infectious agents should be minimized by handwashing by attendants, meticulous care of the intravenous catheter, and assignment to an unshared room. Raw seafood

and exposure to plants should be avoided.

Remission Induction Therapy

AML Variants Other than APL

- Cytotoxic chemotherapy is based on the concept that the marrow contains two competing populations of stem cells (leukemic monoclonal and normal polyclonal) and that profound suppression of the leukemic cells, such that they can no longer be detected morphologically in marrow aspirates or biopsies, is necessary in order to permit recovery of normal hematopoiesis.
- Therapy is usually initiated with two drugs, including an anthracycline antibiotic or anthraquinone and cytosine arabinoside (**Table 45–7**).
- Rate of remission is inversely correlated with a patient's age and cytogenetic risk category. Those who have AML induced by prior chemotherapy or radiotherapy, and those who evolved to AML from an antecedent predisposing chronic myeloid neoplasm, have lower remission rates than patients with de novo AML.
- Patients with leukocyte counts of 100×10^9/L or more should be treated to achieve more rapid cytoreduction using hydroxyurea 1.5 to 2.0 g, orally, every 6 hours for about 36 hours with downward titration of dose as blast count diminishes. Leukapheresis (coupled with hydroxyurea) can further accelerate reduction of the white cell count (see Chap. 93). Hydration sufficient to maintain urine flow of at least 100 mL/h is necessary during the first several days of cytotoxic therapy.
- Severe neutropenia and other factors resulting from cytotoxic therapy frequently result in infection and require cultures and rapid institution of broad-spectrum antibiotic therapy until cultures results are known. If an organism is identified, antibiotic therapy can be tailored to its sensitivity spectrum.
- Red cell and platelet transfusions are often required. Patients should receive leukoreduced blood products to avoid allergic reactions and allosensitization and those who are candidates for allogeneic hematopoietic stem cell transplantation should receive irradiated blood products.
- Patients with evidence of intravascular coagulation or excessive fibrinolysis should be treated for those conditions (see Chaps. 85 and 86). If findings are equivocal, monitor patients with plasma fibrinogen, D-dimer, and coagulation assays. These complications are of highest prevalence in patients with APL and acute monocytic leukemia, but may occur uncommonly with other subtypes.

TABLE 45–7 REMISSION INDUCTION FOR ACUTE MYELOGENOUS LEUKEMIA: EXAMPLES OF CYTOSINE ARABINOSIDE AND ANTHRACYCLINE ANTIBIOTIC COMBINATIONS

Cytarabine	Anthracycline Antibiotic ± Another Agent	No. of Patients	Age Range in Years (Median)	Complete Remissions (%)	Year of Report
100 mg/m^2, days 1–7	DNR 50 mg/m^2, days 1–5	407	15–64 (47)	77.5	2011
100 mg/m^2, days 1–7	IDA 12 mg/m^2, days 1–3	525	15–64 (47)	78.2	2011

100 mg/m^2, days 1–7	DNR 45 mg/m^2, days 1–3	330	17–60 (47)	57	2009
100 mg/m^2, days 1–7	DNR 90 mg/m^2, days 1–3	327	18–60 (48)	71	2009
200 mg/m^2, days 1–7	DNR 60 mg/m^2, days 1–3	200	16–60 (45)	72	2004
200 mg/m^2, days 1–7	DNR 60 mg/m^2, days 1–3 Cladribine 5 mg/m^2, days 1–5	200	16–60 (45)	69	2004
200 mg/m^2 twice per day for 10 days (some in this report received FLAG-IDA vs H-DAT)	DNR 50 mg/m^2, days 1, 3, 5 Thioguanine 100 mg/m^2 twice per day, days 10–20 Gemtuzumab ozogamicin 3 mg/m^2, day 1	64	18–59 (46.5)	91	2003
3 g/m^2 every 12 h for 8 doses	60 mg/m^2 DNR daily for 2 days	122	Adults	80	2000
100 mg/m^2 daily for 7 days (2 courses always given)	IDA 12 mg/m^2 daily for 3 days	153	NR	63	2000
500 mg/m^2 by continuous infusion, days 1–3, 8–10	Mitoxantrone 12 mg/m^2 for 3 days Etoposide 200 mg/m^2 days 8–10	133	15–70 (43)	60	1996
100 mg/m^2 daily for 7 days	DNR 45 mg/m^2 for 3 days	113	NR (55)	59	1992
100 mg/m^2 daily for 7 days	IDA 13 mg/m^2 for 3 days	101	NR (56)	70	1992

DNR, daunorubicin; FLAG, fludarabine, cytarabine, and granulocyte colony-stimulating factor; H-DAT, hydroxydaunorubicin, cytarabine, and thioguanine; IDA, idarubicin; NR, not reported.

All drugs are administered intravenously, except for thioguanine, which is administered orally. The reader is advised to consult the original reports for details of induction, consolidation or continuation therapy, and ancillary therapy.

Source: *Williams Hematology*, 9th ed, Chap. 88, Table 88–6.

APL Treatment

- The use of all-*trans* retinoic acid (ATRA) has decreased the frequency of the hemorrhagic complications of APL. However, the lethal complication of intracerebral hemorrhage still occurs in approximately 10% of patients, despite effective therapy. ATRA should be started urgently on the suspicion that the acute leukemia is APL and then either continued or discontinued when a determination if the *RARa* gene on chromosome 17 is rearranged by fluorescence in situ hybridization analysis or polymerase chain reaction measurement.
- Arsenic trioxide coupled with ATRA is an effective regimen in lower risk patients (white cell count less than 10 × 10^9/L) (**Table 45–7**).
- See **Table 45–8** for several treatment protocols used for patients with higher or lower risk APL.

TABLE 45–8 EXAMPLES OF TREATMENT PROTOCOLS FOR ACUTE PROMYELOCYTIC LEUKEMIA

Induction	Consolidation
High-risk patients	
ATRA 45 mg/m^2 PO in divided doses Daunorubicin 60 mg/m^2 IV for 3 days	1st cycle: Daunorubicin 60 mg/m^2 IV for 3 days; cytarabine 200 mg/m^2 IV for 7 days

Cytarabine 200 mg/m^2 IV for 7 days	2nd cycle: Cytarabine 2 g/m^2 (or 1.5 g/m^2 in older patients) IV, every 12 hours for 5 days plus daunorubicin 45 mg/m^2 IV for 3 days
ATRA 45 mg/m^2 PO (days 1–36 in divided doses) Idarubicin (6–12 mg/m^2 based on age) IV on days 2, 4, 6, and 8 Arsenic trioxide 0.15 mg/kg IV (days 9–26)	1st cycle: ATRA 45 mg/m^2 PO in divided doses for 28 days; arsenic trioxide 0.15 mg/kg IV per day for 28 days 2nd cycle: ATRA 45 mg/m2 PO for 7 days every 2 weeks × 3; arsenic trioxide 0.15 mg/kg per day × 5 days IV for 5 weeks
Low-risk patients	
ATRA 45 mg/m^2 PO in divided doses daily until remission; arsenic trioxide 0.15 mg/kg IV daily until remission	Arsenic trioxide 0.15 mg/kg IV per day, 5 days per week for 4 weeks every 8 weeks for 4 cycles ATRA 45 mg/m^2 PO per day for 2 weeks every 4 weeks for 7 cycles
ATRA 45 mg/m^2 PO in divided doses until clinical remission; idarubicin 12 mg/m^2 IV on days 2, 4, 6, and 8	1st cycle: ATRA 45 mg/m^2 PO for 15 days; idarubicin 5 mg/m^2 IV for 4 days 2nd cycle: ATRA 45 mg/m^2 PO for 15 days; mitoxantrone 10 mg/m^2 IV for 5 days 3rd cycle: ATRA 45 mg/m^2 PO for 15 days; idarubicin 12 mg/m^2 IV for 1 dose

IV, intravenously; PO, orally.
Note: The reader is advised to consult the original reference in *Williams Hematology*, 9th edition, for details of the administration of the chemotherapy regimens. "High risk" is defined as a white cell count at diagnosis ≥10 × 10^9/L. "Low risk" is defined as a white cell count at diagnosis < 10 × 10^9/L.
Source: *Williams Hematology*, 9th ed, Chap. 88, Table 88–8.

Remission Maintenance Therapy

- Intensive postremission therapy results in longer duration of remission.
- There is no agreement on best current postremission therapy, in part because the age, cytogenetic alteration, morphologic subtype, and other factors may dictate different options.
- Three principal modalities are available:
  — Intensive cytotoxic drug therapy (eg, high-dose cytarabine)
  — Very intensive chemotherapy and autologous stem cell infusion
  — Pretransplant conditioning regimen (eg, chemoradiation) followed by allogeneic hematopoietic stem cell transplantation in patients deemed suitable for this approach based on availability of a suitable donor, age, comorbidities, probability of a salutary outcome, the patient's agreement, and other factors evaluated by the responsible transplant physician (see Chap. 39)

Relapsed or Refractory Patients

- Chemoradiation followed by allogeneic stem cell transplantation in patients with AML in second remission can induce long-term survival in about 25% so treated. Treatment with chemotherapy alone in this group is unlikely to result in long-term remission.
- Patients who relapse more than 12 months after initial chemotherapy can be given the same treatment again.
- Another therapy for relapsed patients is high-dose cytosine arabinoside with or without additional drug(s), such as mitoxantrone, amsacrine, or etoposide (**Table 45–9**).

- Patients with APL who relapse may respond to arsenic trioxide treatment and chemotherapy.
- Chemoradiation followed by allogeneic hematopoietic stem cell transplantation may be used in refractory patients. Approximately 10% of such AML patients may be cured, and approximately 25% percent may achieve remissions of at least 3 years with marrow transplantation.

TABLE 45–9 EXAMPLES OF CHEMOTHERAPY USED FOR RELAPSED OR REFRACTORY PATIENTS

Regimen	No. of Patients	Percent of Patients Entering a Complete Remission (Median Duration)	Year Results Published
Clofarabine 40 mg/m^2, IV, days 1–5	163	35.2 (6.6 months)	2012
Cytarabine 1 g/m^2, IV, days 1–5	163	17.8 (6.3 months)	2012
Clofarabine 25 mg/m2, IV, daily for 5 days Cytarabine 2 g/m^2, IV, daily for 5 days G-CSF 5 mcg/kg per day subcutaneously daily until ANC ≥2.0 × 10^9/L	50	46 (9 months)	2011
Gemtuzumab ozogamicin 6 mg/m^2, IV, days 1 and 13 Idarubicin 12 mg/m^2, IV, days 2–4 Cytarabine 1.5 g/m^2, IV, days 2–5	15	21 (27 weeks)	2003
Mitoxantrone 12 mg/m^2, IV, days 1–3 Cytarabine 500 mg/m^2, IV, days 1–3 Followed (at blood count recovery) by: Etoposide 200 mg/m^2, IV, days 1–3 Cytarabine 500 mg/m^2, IV, days 1–3	66	36 (5 months)	2003
Cladribine 5 mg/m^2, IV, days 1–5 Cytarabine 2 g/m^2, IV, days 1–5, 2 h after cladribine G-CSF 10 mcg/kg subcutaneously, each day, days 1–5	58	50 (29% disease-free at 1 year)	2003
Fludarabine 30 mg/m^2, IV, days 1–5 Cytarabine 2 g/m^2, IV, days 1–5 Idarubicin 10/m^2, IV, days 1–3 G-CSF 5 mcg/kg subcutaneously each day, up to 6 doses until neutrophil recovery	46	52 (13 months)	2003
Gemtuzumab ozogamicin 9 mg/m^2, IV, days 1 and 15	43	9	2002
Mitoxantrone 4 mg/m^2, IV, days 1–3 Etoposide 40 mg/m^2, IV, days 1–3 Cytarabine 1 g/m^2, IV, days 1–3, ± valspodar (PSC-833)	37	32	1999
Fludarabine 30 mg/m^2, IV, days 1–5 Cytarabine 2 g/m^2, IV, days 1–5± idarubicin 12 mg/m2, IV, days 1–3 G-CSF 400 mcg/m^2, subcutaneously, daily until complete remission	85	66	1995

ANC, absolute neutrophil count; G-CSF, granulocyte colony-stimulating factor; IV, intravenously.

Note: The reader is advised to consult the original reference in *Williams Hematology*, 9th edition, for details of chemotherapy regimen administration.

Source: *Williams Hematology*, 9th ed, Chap. 88, Table 88–7.

Special Therapeutic Consideration

- It may be necessary to reduce the dose of cytotoxic drugs administered to patients older than 60 years of age if concurrent illnesses (eg, heart disease, diabetes) or other factors so dictate.
- Treatment of pregnant patients in the first trimester with antimetabolites increases the risk of congenital anomalies in the infant. However, babies born after intensive chemotherapy administered in the late second and third trimesters may develop normally.
- Intensive multidrug chemotherapy has been used successfully in patients younger than 17 years of age in whom long-term remission rates are in the 50% range. Infants younger than 1 year of age with neonatal AML do not respond well to chemotherapy and should be considered for allogeneic hematopoietic stem cell transplantation.

Special Nonhematopoietic Adverse Effects of Treatment

- Skin rashes develop in more than 50% of the patients with AML during chemotherapy, often caused by one of the following drugs: allopurinol, β-lactam antibiotics, cytosine arabinoside, trimethoprim-sulfamethoxazole, miconazole, and ketoconazole.
- Cardiomyopathy may develop in patients receiving anthracycline antibiotics and other agents. The adverse effect on the heart may be quite delayed developing decades after therapy.
- Systemic candidiasis syndrome presents with fever, abdominal pain, and hepatomegaly and is associated with multiple hepatic candidiasis lesions detected radiographically or by ultrasound. Patients may respond to prolonged therapy with antifungal agents, such as azoles or echinocandins.
- Patients receiving intensive cytotoxic therapy may develop necrotizing inflammation of the cecum (typhlitis), which can simulate appendicitis. This may require surgical intervention.
- Fertility may be sustained or be recovered in men and women undergoing intensive cytotoxic therapy, but infertility can occur especially after intensive regimens used for hematopoietic stem cell transplantation.

Results of Treatment

- By using current therapeutic approaches, remission rates approach 90% in children, 70% in young adults, 60% in the middle aged, and 25% in older patients.
- Median survival of all patients in the full age-range is about 18 months because of the high proportion of patients older than 65 years of age. The influence of age on survival of patients with AML is shown in **Table 45–10**.
- Relapse or development of a new leukemia has occurred rarely after 8 years in adults and 16 years in children.

TABLE 45–10 **ACUTE MYELOGENOUS LEUKEMIA: FIVE-YEAR PERCENT RELATIVE SURVIVAL RATES (2004–2010)**

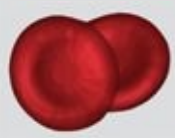
Age (Years)	Acute Myelogenous Leukemia*
< 45	56
45–54	39
55–64	27
65.74	11

> 75	1.8
< 65	43
> 65	6.0

*Percent rounded to nearest integer.
Data from SEER Cancer Statistics. Table 13.6. National Cancer Institute, Washington, DC. Available at: http://seer.cancer.gov/csr/1975_2011/browse_csr.php?sectionSEL=13&pageSEL=sect_13_table.16.html

Features Influencing the Outcome of Therapy

- Both the probability of remission and the duration of response decrease with increasing age at the time of diagnosis.
- Cytogenetic abnormalities such as inv(16), t(16;16), del(16q), t(8;21), or t(15;17) indicate a better prognosis, whereas –5, del(5q), –7, del(7q), t(9;22), and others indicate a poorer prognosis. A normal karyotype, +6, +8, and certain other cytogenetic findings are intermediate in outlook. These are relative prognostic projections, and a better prognosis may not be an excellent prognosis.
- Specific gene mutations such as those involving *FLT3* may modify prognosis (see **Table 45–5**).
- Prognosis may also influence the decision to use allogeneic hematopoietic stem cell transplantation in first remission.
- AML that develops after prior cytotoxic therapy for another disease or after a clonal cytopenia or oligoblastic leukemia (myelodysplastic syndromes) has a significantly lower remission rate and shorter remission duration on average than de novo AML.
- A leukocyte count greater than 30×10^9/L or a blast cell count greater than 15×10^9/L decreases the probability and the duration of remission.
- Many other laboratory findings are correlated with decreased remission rate or duration (see *Williams Hematology*, 9th ed, Table 89–11).



For a more detailed discussion, see Jane L. Liesveld and Marshall A. Lichtman: Acute Myelogenous Leukemia, Chap. 88 in *Williams Hematology*, 9th ed.

CHAPTER 46

The Chronic Myelogenous Leukemias

BCR-ABL1-POSITIVE CHRONIC MYELOGENOUS LEUKEMIA

- *BCR-ABL1*–positive chronic myelogenous leukemia (CML) results from a somatic mutation in a pluripotential lymphohematopoietic cell, yielding a fusion oncogene (*BCR-ABL1*).
- CML is characterized by granulocytic leukocytosis, granulocytic immaturity, basophilia, anemia, and often thrombocytosis in the blood, intense leukemic granulocytic precursor expansion in the marrow, and splenomegaly.
- The natural history of the disease is to evolve into an accelerated phase in which cytopenias develop and response to chronic phase therapy is lost; either the chronic or accelerated phase can undergo further clonal evolution to acute leukemia.
- This natural history has been modified significantly by the introduction of inhibitors of the constitutive kinase activity of the BCR-ABL1 oncoprotein.

ETIOLOGY

- Exposure to high-dose ionizing radiation increases the incidence of *BCR-ABL1*–positive CML, with a mode of increased incidence that ranges from 4 to 11 years in different exposed populations.
- Obesity may be an endogenous risk factor.

PATHOGENESIS

Genetic Abnormality

- *BCR-ABL1*–positive CML is the result of an acquired genetic abnormality that induces a malignant transformation of a single pluripotential lymphohematopoietic cell.
- The proximate cause is a translocation between chromosome 9 and 22 [t(9;22)]. This alteration juxtaposes a portion of the *ABL* proto-oncogene from chromosome 9 to a portion of the *BCR* gene on chromosome 22.
- The resulting gene fusion, *BCR-ABL1*, creates an oncogene that encodes an elongated protein tyrosine phosphokinase (usually p210) that is constitutively expressed. This mutant protein disrupts cell signal pathways and results in the malignant transformation.
- The genetic alteration is present in erythroid, neutrophilic, eosinophilic, basophilic, monocytic, megakaryocytic, and marrow B- and T-lymphocytic cells, consistent with its origin in a pluripotential lymphohematopoietic cell.
- The designation Philadelphia (Ph) chromosome specifically refers to chromosome 22 with a

shortened long arm (22q-) and is evident by light microscopy of cell metaphase preparations in approximately 90% of cases. Fluorescence in situ hybridization (FISH) can identify the fusion *BCR-ABL1* gene in approximately 96% of cases. Approximately 4% of cases with a blood and marrow phenotype indistinguishable from *BCR-ABL1* CML do not have rearrangement in the *BCR* gene.

Hematopoietic Abnormalities

- There is a marked expansion of granulocytic progenitors and a decreased sensitivity of the progenitors to regulation resulting in an inexorable increase in white cell count and decrease in hemoglobin concentration.
- Megakaryocytopoiesis is often expanded. Erythropoiesis is usually moderately deficient.
- Function of the neutrophils and platelets is nearly normal; infection and bleeding are not a feature of the chronic phase.

EPIDEMIOLOGY

- *BCR-ABL1*–positive CML accounts for approximately 15% of all cases of leukemia and approximately 3% of childhood leukemias in the United States.
- Males are affected at approximately 1.5 times the rate of females.
- The age-specific incidence rate increases exponentially from late adolescence (0.2 cases/100,000) to octogenarians (10 cases/100,000).
- Familial occurrence is vanishingly rare, and there is no concordance in identical twins.
- Neither chemical agents, including benzene, cytotoxic drugs, nor combusted tobacco smoke has a causal relationship with CML.

CLINICAL FEATURES

- Approximately 30% of patients are asymptomatic at the time of diagnosis. The disease is discovered coincidentally when an elevated white cell count is noted at a medical evaluation.
- Symptoms are gradual in onset and may include easy fatigability, malaise, anorexia, abdominal discomfort and early satiety, weight loss, and excessive sweating.
- Less frequent symptoms are those of hypermetabolism, such as night sweats, heat intolerance, and weight loss, mimicking hyperthyroidism; gouty arthritis; priapism, tinnitus, or stupor from leukostasis as a consequence of hyperleukocytosis; left upper quadrant and left shoulder pain because of splenic infarction; diabetes insipidus; and urticaria as a result of histamine release.
- Physical signs may include pallor, splenomegaly, and sternal tenderness.

LABORATORY FEATURES

Blood Findings

- The hemoglobin concentration is decreased in most patients at the time of diagnosis. Occasional nucleated red cells can be seen on the stained blood film. Rare patients may have a

normal or slightly increased hematocrit at the time of presentation.

- The leukocyte count is elevated, usually above 25 × 10^9/L, and often above 100 × 10^9/L (see **Figure 46–1**). Granulocytes at all stages of development are present in the blood, but segmented and band neutrophils predominate (see **Table 46–1** and **Figure 46–2**). Hypersegmented neutrophils are often present.
- An increase in the absolute basophil count is found in virtually all patients. Basophils usually constitute less than 10% of leukocytes in the chronic phase but occasionally make up a higher proportion. The absolute eosinophil count may also be increased.
- The platelet count is normal or increased at diagnosis but may increase during the course of the chronic phase, sometimes reaching 1000 × 10^9/L and uncommonly as high as 5000 × 10^9/L (see **Figure 46–1**).
- Neutrophil alkaline phosphatase activity is low or absent in more than 90% of patients. It may also be low in paroxysmal nocturnal hemoglobinuria, hypophosphatasia, with androgen therapy, and in about 25% of patients with primary myelofibrosis. It has been largely replaced as a diagnostic marker by cytogenetic and molecular analysis (see below).
- Whole blood histamine is markedly increased (mean = 5000 ng/mL) compared with normal levels (mean = 500 ng/mL) and is correlated with the absolute basophil count. Occasional cases of pruritus, urticaria, and gastric hyperacidity occur.

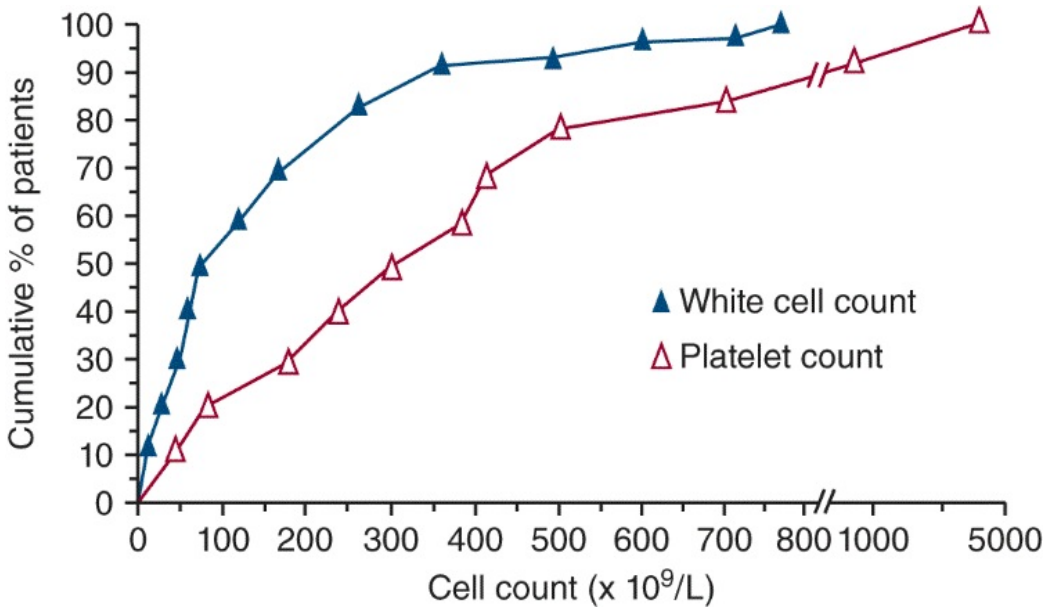


FIGURE 46–1 Total white cell count and platelet count of 90 patients with CML at the time of diagnosis. The cumulative percent of patients is on the *ordinate*, and the cell count is on the *abscissa*. Fifty percent of patients had a white cell count greater than 100 × 10^9/L and a platelet count greater than approximately 300 × 10^9/L at the time of diagnosis. (Hematology Unit, University of Rochester Medical Center. Source: *Williams Hematology*, 9th ed, Chap. 89, Fig. 89–6.)

TABLE 46–1 **WHITE BLOOD CELL DIFFERENTIAL COUNT AT THE TIME OF DIAGNOSIS IN 90 CASES OF PHILADELPHIA-CHROMOSOME–POSITIVE CHRONIC MYELOGENOUS LEUKEMIA**

Cell Type	Percent of Total Leukocytes (Mean Values)
Myeloblasts	3
Promyelocytes	4
Myelocytes	12

Metamyelocytes	7
Band forms	14
Segmented forms	38
Basophils	3
Eosinophils	2
Nucleated red cells	0.5
Monocytes	8
Lymphocytes	8

Note: In these 90 patients, the mean hematocrit was 31 mL/dL, mean total white cell count was 160 × 10^9/L, and mean platelet count was 442 × 10^9/L at the time of diagnosis (Hematology Unit, University of Rochester Medical Center). More recent studies indicate the blood blast count may be zero at diagnosis, presumably because of earlier diagnosis from more frequent medical surveillance.
Source: *Williams Hematology,* 9th ed, Chap. 89, Table 89–1.

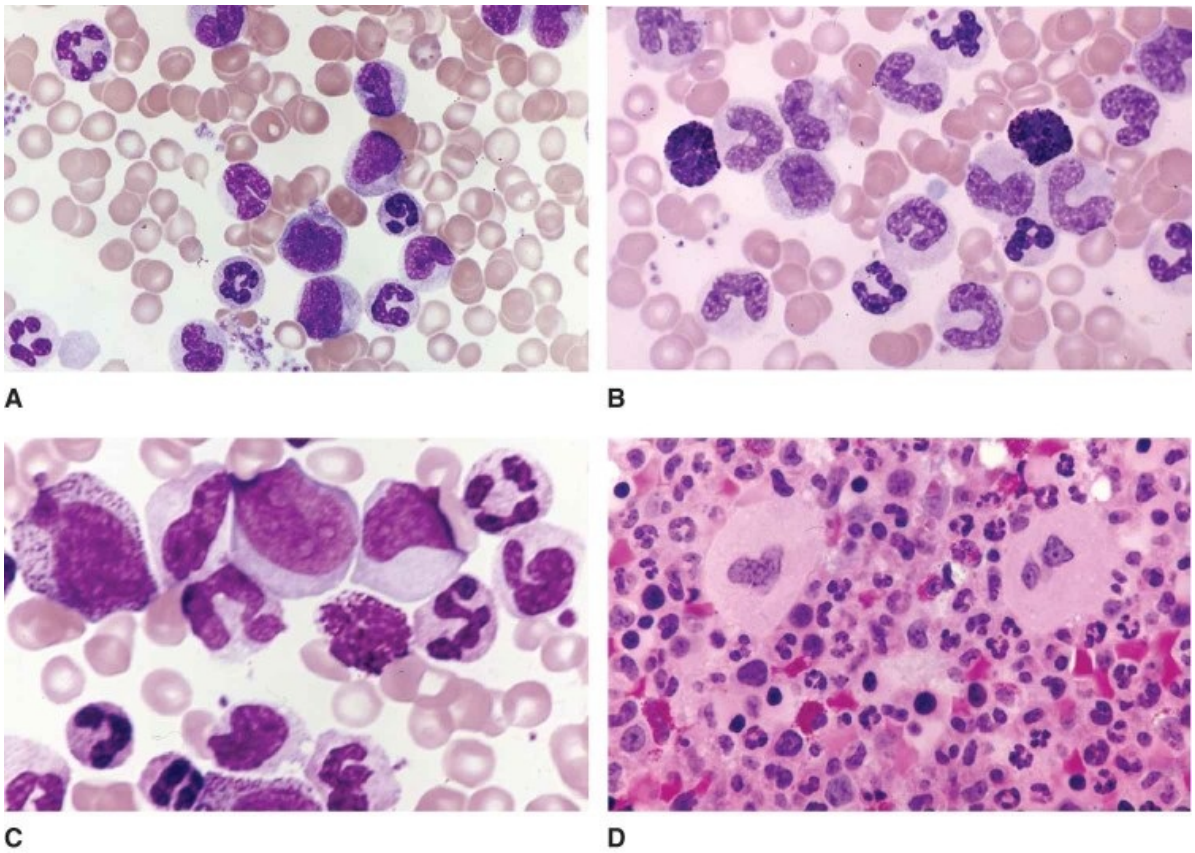


FIGURE 46–2 Blood and marrow cells characteristic of CML. **A.** Blood film. Elevated leukocyte count. Elevated platelet count (aggregates). Characteristic array of immature (myelocytes, metamyelocytes, band forms) and mature neutrophils. **B.** Blood film. Elevated leukocyte count. Characteristic array of immature (myelocytes, metamyelocytes, band forms) and mature neutrophils. Two basophils in the field. Absolute basophilia is a constant finding in CML. **C.** Blood film. Elevated leukocyte count. Characteristic array of immature (promyelocytes, myelocytes, metamyelocytes, band forms) and mature neutrophils. Basophil in the field. Two myeloblasts in upper center. Note multiple nucleoli (abnormal) and agranular cytoplasm. **D.** Marrow section. Hypercellular. Replacement of fatty tissue (normally approximately 60% of marrow volume in adults of this patient's age) with hematopoietic cells. Intense granulopoiesis and evident megakaryocytopoiesis. Decreased erythropoiesis. (Reproduced with permission from *Lichtman's Atlas of Hematology,* www.accessmedicine.com.)

Marrow Findings and Cytogenetics

- The marrow is markedly hypercellular, primarily because of granulocytic hyperplasia.

Megakaryocytes may be increased in number. Occasionally, sea-blue histiocytes and macrophages engorged with glucocerebroside from exaggerated cell turnover may be present. The latter cells mimic the appearance of Gaucher cells (pseudo-Gaucher cells).

- Reticulin fibrosis rarely is prominent in the marrow and is correlated with the expansion of megakaryocytes.
- The Ph chromosome is present in metaphase preparations of marrow cells in approximately 90% of patients. Some of the remaining patients have variant or cryptic translocations (see **Figure 46–3**). FISH identification of *BCR-ABL1* fusion and reverse transcriptase polymerase chain reaction (RT-PCR) to detect *BCR-ABL1* mRNA transcripts are the two most sensitive diagnostic tests used.
- Molecular evidence of the *BCR-ABL1* fusion gene is present in the blood and marrow cells of virtually all patients (96%) with apparent CML. The remainder has a phenotype indistinguishable from CML but no evidence of *BCR-ABL1* (see "Philadelphia Chromosome–Negative CML," below).

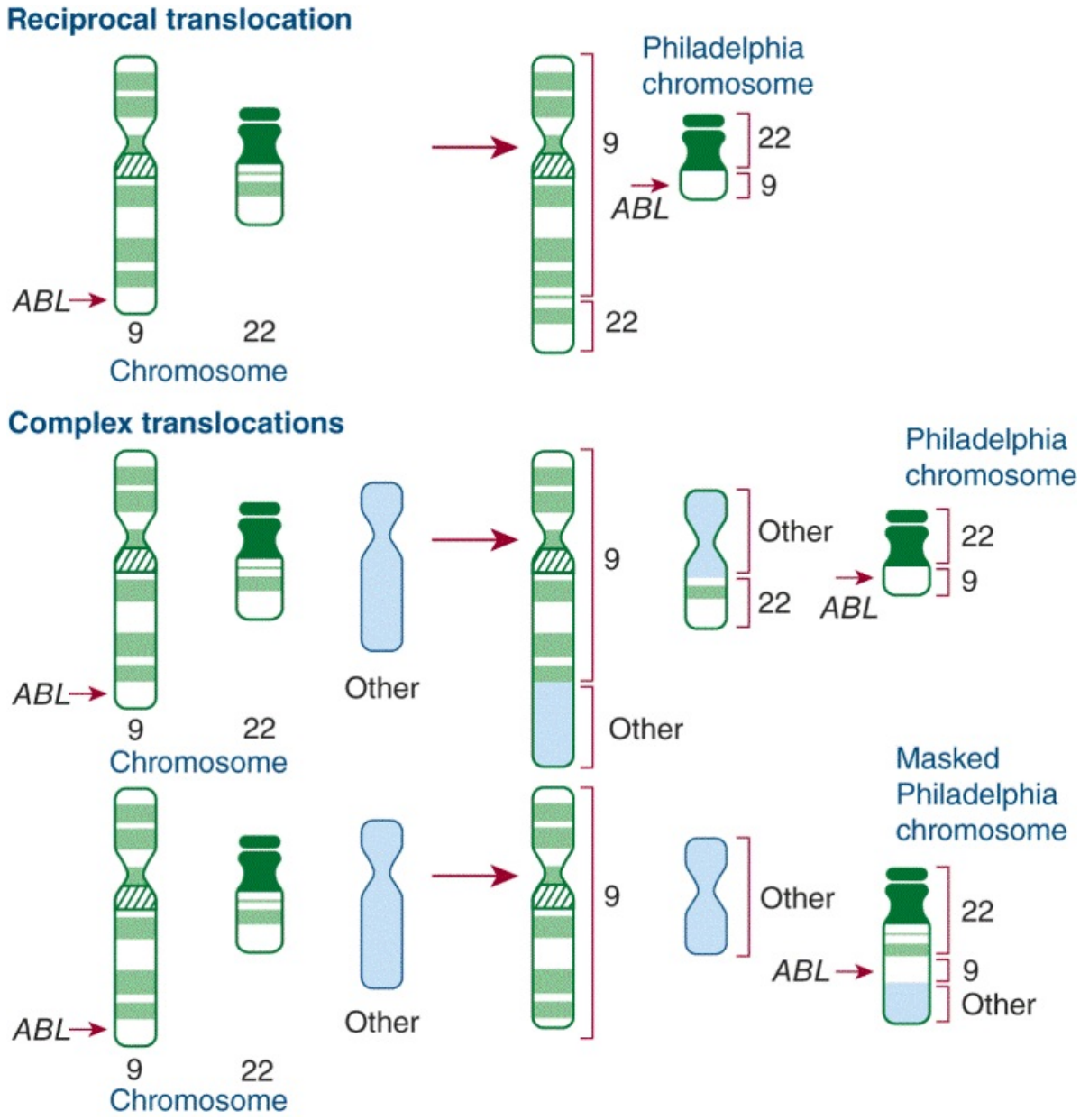


FIGURE 46–3 Translocations involved in chronic myelogenous leukemia. The positions of the *ABL* gene in each of the chromosomes before and after the translocation are noted. The origin of the chromosomal segments in each of the translocated

chromosomes is indicated by a *bracket* on the side of the chromosome. (Reproduced with permission from Rosson D, Reddy EP: Activation of the abl oncogene and its involvement in chromosomal translocations in human leukemia, *Mutat Res* 1988 May;195(3):231-243.)

Serum Findings

- Hyperuricemia (and hyperuricosuria) are frequent.
- Serum lactic acid dehydrogenase (LDH) activity is elevated.
- Serum vitamin B_{12}-binding protein and serum vitamin B_{12} levels are increased in proportion to the total leukocyte count.
- Pseudohyperkalemia may be a result of the release of potassium from granulocytes in clotted blood, and spurious hypoxemia and hypoglycemia may result from cellular utilization in blood under laboratory analysis.

SPECIAL CLINICAL FEATURES

Hyperleukocytosis

- Approximately 15% of patients present with leukocyte counts of 300 × 10^9/L (see **Figure 46–1**) or higher and may have signs and symptoms of leukostasis from impaired microcirculation in the lungs, brain, eyes, ears, or penis.
- Patients may have tachypnea, dyspnea, cyanosis, dizziness, slurred speech, delirium, stupor, visual blurring, diplopia, retinal vein distention, retinal hemorrhages, papilledema, tinnitus, impaired hearing, or priapism.

Philadelphia Chromosome–Positive or *BCR-ABL1*–Positive Thrombocythemia

- Some patients (approximately 5%) present with the features of essential thrombocythemia (elevated platelet count and megakaryocytosis in marrow without significant change in hemoglobin or white cell count) but have the Ph chromosome and *BCR-ABL1* fusion gene or no Ph chromosome but a *BCR-ABL1* gene rearrangement.
- These patients later develop clinical CML or blast crisis. This presentation is considered a forme fruste of CML.

Neutrophilic CML

- This is a rare variant of *BCR-ABL1*–positive CML in which the leukocytosis is composed of mature neutrophils.
- The white cell count is lower on average (30 to 50 × 10^9/L), blood basophilia and myeloid immaturity are absent, and splenic enlargement may not be evident.
- These patients have an unusual breakpoint in the *BCR* gene between exons 19 and 20, which leads to a larger BCR-ABL1 fusion oncoprotein (230-kDa compared to the classic 210-kDa fusion protein).
- This variant tends to have an indolent course, perhaps because of the very low levels of p230 in the cells.

Minor BCR Breakpoint–Positive CML

- This variant of CML has the BCR breakpoint at the first intron, resulting in a 190-kDa BCR-ABL1 fusion protein compared with the classic 210-kDa fusion protein. In this variant, the white cell count is lower on average, monocytosis is seen, and basophilia and splenomegaly are less prominent.
- This variant usually progresses to acute leukemia more rapidly on average than in classical CML.

DIFFERENTIAL DIAGNOSIS

- The diagnosis of CML is made on the basis of neutrophilic granulocytosis with some immature cells (promyelocytes, myelocytes), basophilia, and splenomegaly, coupled with detection of the Ph chromosome and/or the *BCR-ABL1* fusion gene.
- Polycythemia vera is usually distinguished by the elevated hemoglobin concentration, white cell count less than 25×10^9/L, and absence of myeloid immaturity in the blood film. Virtually all patients have a mutation in the *JAK2* gene (see Chap. 41).
- Primary myelofibrosis has distinctive red cell morphologic changes (poikilocytosis, anisocytosis, and teardrop red cells), invariable splenomegaly, and usually reticulin fibrosis on marrow biopsy. Mutations in *JAK2*, *CALR*, or *MPL* are present in 85% of patients (see Chap. 47).
- Essential thrombocythemia rarely has a white cell count greater than 25×10^9/L. *JAK2* mutations are found in approximately 50% of patients and *BCR-ABL1* is absent (see Chap. 42).
- In each of the other chronic clonal myeloid diseases, the absence of *BCR-ABL1* is a key distinction from CML.
- Extreme reactive leukocytosis (leukemoid reaction) may occur in patients with an inflammatory disease, cancer, or infection, but is not associated with basophilia, significant granulocytic immaturity, or splenomegaly. The clinical setting usually distinguishes a leukemoid reaction. *BCR-ABL1* oncogene is absent.

TREATMENT

Hyperuricemia

- Most patients have massive expansion and turnover of blood cells with accompanying hyperuricemia or the risk of hyperuricemia with therapeutic cytoreduction.
- Patients should receive allopurinol, 300 mg/d orally, and adequate hydration before and during therapy to control exaggerated hyperuricemia and hyperuricosuria. It takes several days to lower the uric acid level. Discontinue the drug when uric acid is under control to minimize toxicity, especially skin rashes.
- Rasburicase, a recombinant urate oxidase, is effective in hours but is given intravenously and is more expensive. The manufacturer recommends 5 days of use but that is usually unnecessary to lower the uric acid sufficiently. If the uric acid is very high (eg, > 9 mg/dL), a single dose of 0.2 mg/kg of ideal body weight can be used with allopurinol on successive days for approximately 36 hours, dependent on response.

Hyperleukocytosis

- If the white cell count is very high (> 300 × 10^9/L), especially if signs of hyperleukocytosis are present, hydroxyurea, 1 to 6 mg/d, depending on the height of the white cell count may be used initially. The dose is decreased as the count decreases and is usually at about 0.5 to 1 g/d when the leukocyte count reaches 20 × 10^9/L.
- If needed, maintenance doses should be adjusted to keep the total white count at about 5 to 10 × 10^9/L.
- Blood counts should be followed and the drug should be stopped if the white count falls to 5 × 10^9/L or less.
- The major side effect of hydroxyurea is suppression of hematopoiesis, often with megaloblastic erythropoiesis.
- Hyperleukocytosis usually responds rapidly to hydroxyurea but, if necessary, because of compelling signs of hyperleukocytosis (eg, stupor, priapism), leukapheresis can be instituted simultaneously. Leukapheresis removes large numbers of cells minimizing the metabolic effects of tumor lysis (eg, exaggerating hyperuricemia, hyperphosphatemia) while hydroxyurea is killing and retarding production of leukemic cells.

Thrombocytosis

- In the uncommon circumstance in which thrombocytosis is so prominent that early platelet reduction therapy is warranted, hydroxyurea or anagrelide can be used. Plateletpheresis and hydroxyurea can be combined if there are acute vascular problems.

Tyrosine Kinase Inhibitor Therapy

- Any of three established tyrosine kinase inhibitors (TKIs) can be used for the initial treatment of CML: imatinib mesylate (imatinib), dasatinib, or nilotinib. These are administered orally. Although the latter two agents have a significant, approximately 15%, increase in cytogenetic and molecular response and a more rapid response on average when compared to imatinib mesylate, an advantage of the latter two agents, as judged by overall survival, has not been established. If a patient has had an excellent molecular response on imatinib, it is reasonable to continue that therapy. Because of the advantages projected for dasatinib or nilotinib, they are often used for initial therapy. A table summarizing the comparative features of imatinib, nilotinib, and dasatinib are shown in **Table 46–2**.
- Patients with newly diagnosed CML should be started on one of the TKIs. Dasatinib, 100 mg/d orally, nilotinib, 300 mg every 12 hours, orally, or imatinib 400 mg/day, orally, are approved by the US Food and Drug Administration. In cases with hyperleukocytosis, white cell reduction should precede the start of a TKI to lower the risk of tumor lysis syndrome (see “Hyperleukocytosis,” above).
- The efficacy of a TKI is measured by three indicators of response: hematologic, cytogenetic, and molecular (see **Table 46–3**).
- The guidelines for the tests used to measure the response to TKI therapy are shown in **Table 46–4**.
- The criteria for assessing the response to a TKI are shown in **Table 46–5**.
- As long as a patient is having continued reduction in the size of the leukemic clone as judged

by cytogenetic or molecular measurements, the TKI is continued at the same dose.

- If the patient stops responding to a TKI before a complete cytogenetic or complete molecular remission occurs, the dose may be increased and/or an alternative TKI should be considered (eg, nilotinib or dasatinib).
- Imatinib is generally well tolerated. The main side effects are fatigue, edema, nausea, diarrhea, muscle cramps, and rashes. Severe periorbital edema is occasionally observed. Hepatotoxicity occurs in about 3% of patients. There are numerous other uncommon side effects. The principal side effects of dasatinib include cytopenias and fluid retention, notably pleural effusion. The side effects of nilotinib include rash, hyperglycemia, increased serum lipase and amylase (pancreatitis), transaminitis, and hypophosphatemia (see **Table 46–2**).
- Neutropenia and thrombocytopenia may occur early in TKI use. Dose reduction for side effects is not recommended. If absolutely necessary, cessation may be required. Often, the mild cytopenias improve with continued therapy.
- The definition of the response to TKI therapy is shown in **Table 46–5**. Duration of response may extend over years or decades.
- TKIs may be teratogenic. Women in the childbearing age group can (1) use contraception during therapy; (2) use interferon-α until delivery, if pregnant when diagnosed, and then be placed on a TKI; or (3) if in a complete molecular remission, could have TKI therapy stopped until conception and then be restarted after delivery.
- Leukapheresis may be useful as sole treatment in patients in the first trimester when it may be necessary to control the white cell count and splenic enlargement without chemotherapy.
- In the case of TKI intolerance or resistance, an alternative TKI can be tried.
- Failure of TKI therapy during the course of the disease is often the result of a mutation in the *ABL* portion of *BCR-ABL1*, which interferes with drug action.
- There are several mutations that can induce TKI resistance. Thus, PCR is impractical as a tool to find mutations. It is possible to sequence *ABL* and, by comparing the results to known mutations, determine the likelihood that a TKI-sensitive mutation may be present.
- If the T315I *ABL* mutation is found, ponatinib may be considered (see **Table 46–2**). Allogeneic hematopoietic cell transplantation, also, should be considered for eligible patients.

TABLE 46–2 COMPARISON OF TYROSINE KINASE INHIBITORS

	Imatinib (Gleevec)	Nilotinib (Tasigna)	Dasatinib (Sprycel)	Bosutinib (Bosulif)	Ponatinib (Iclusig)
Indications	First-line therapy (CP, AP, BP); relapsed/ refractory Ph+ ALL	First-line therapy (CP), resistance or intolerance to imatinib (CP and AP)	First-line therapy (CP), resistance or intolerance to other TKIs (CP, AP, or BP); Ph+ ALL with resistance or intolerance to prior therapy	Second-line therapy (CP, AP, BP with resistance or intolerance)	Resistance or intolerance to prior TKI or Ph+ ALL resistant or intolerant to all other TKIs; all T315I + cases]
Usual dosing	CP 400 mg/d AP/BP/progression 600–800 mg/d	CP 300 mg twice daily AP/BP 400 mg twice daily	CP 100 mg/d AP/BP 140 mg/d	500 mg/d	45 mg/d
Common toxicities (nonhematologic)	GI disturbance, edema (including periorbital), muscle cramps, arthralgias, Hypophosphatemia, rash	Rash, GI disturbances, elevated lipase, hyperglycemia, low phosphorus, increased LFTs	Edema, pleural effusions, GI symptoms, rash, low phosphorus	GI (diarrhea), rash, edema, fatigue, low phosphorus, elevated LFTs	HBP, rash, GI, fatigue, headache
Other significant toxicities	Elevated LFTs (usually appear in first month); rare cardiac toxicity reported	Peripheral vascular disease, PT prolongation, pancreatitis	Pulmonary arterial hypertension, QTc prolongation		Arterial and venous thrombosis, pancreatitis, liver failure, ocular toxicity, cardiac failure
Drug–drug interactions	CYP3A4 inducers decrease levels CYP3A4 inhibitors may increase levels It is an inhibitor of CYP3A4 and CYP2D6 Pgp substrate	CYP3A4 inhibitors increase levels CYP3A4 inducers may decrease levels Inhibitor of CYP3A4, CYP2C8, CYP2C9, CYP2D6 Induces CYP2B6, CYP2C8, and CYP2C9	CYP3A4 inhibitors increase levels CYP3A4 inducer decrease levels Antacids decrease levels H2 antagonists/ proton pump inhibitors decrease levels	CYP3A inhibitors and inducers may alter levels Acid-reducing medication may lower levels	Strong CYP3A inhibitors increased serum levels
Administration considerations	Taken with food	Taken on empty stomach; avoid food 2 hours before and 2 hours after dose	Can be taken with or without a meal	Taken with food	Taken with and without food
Black Box Warnings	None	QT prolongation and sudden death	None	None	Arterial thrombosis; hepatotoxicity
Other considerations	Approved in pediatric patients (340 mg/m^2/d) in CP	Keep potassium, Mg, calcium, phosphorus repleted	Ascites and pericardial effusion can also occur; has CSF penetration		Has activity with T315I mutations; Available in United States through ARIAD PASS program

ALL, acute lymphocytic leukemia; AP, accelerated phase; BP, blast phase; CP, chronic phase; CSF, cerebrospinal fluid; CYP, cytochrome P450; GI, gastrointestinal; HBP, high blood pressure; LFT, liver function tests; Pgp, P-glycoprotein; PT, prothrombin time; TKI, tyrosine kinase inhibitor.
All information is from the commercial package insert of the TKIs as listed.
Source: *Williams Hematology*, 9th ed, Chap. 89, Table 89–2.

TABLE 46–3 DEFINITION OF A TREATMENT RESPONSE TO A TYROSINE KINASE INHIBITOR

Complete hematologic response (CHR)	White cell count < 10×10^9/L, platelet count < 450×10^9/L, no immature myeloid cells in the blood, and disappearance of all signs and symptoms related to leukemia (including palpable splenomegaly) lasting for at least 4 weeks
Minor cytogenetic response (mCyR)	> 35% of cell metaphases are Philadelphia (Ph) chromosome–positive by cytogenetic analysis of marrow cells
Partial cytogenetic response (pCyR)	1%–35% of cell metaphases are Ph chromosome–positive by cytogenetic analysis of marrow cells
Major cytogenetic response (MCyR)	< 35% of cell metaphases contain the Ph chromosome by cytogenetic analysis of marrow cells
Complete cytogenetic response (CCyR)	No cells containing the Ph chromosome by cytogenetic analysis of marrow cells
Major molecular response (MMR)	*BCR-ABL1/ABL1* ratio < 0.1% or a 3-log reduction in quantitative polymerase chain reaction (qPCR) signal from mean pretreatment baseline value, if International Standard (IS)-based PCR not available
Complete molecular response (CMR)	*BCR-ABL1* mRNA levels undetectable by qPCR with assay sensitivity at least 4.5 logs below baseline (IS)

Source: *Williams Hematology*, 9th ed, Chap. 89, Table 89–3.

TABLE 46–4 GUIDELINES FOR TESTS AND TIMING OF TESTING TO MONITOR PATIENTS IN

	CHRONIC PHASE WHO ARE UNDERGOING TYROSINE KINASE INHIBITOR THERAPY

1. At diagnosis, before starting therapy, obtain Giemsa-banding cytogenetics and measure *BCR-ABL1* transcript numbers by qPCR using marrow cells. If marrow cannot be obtained, use FISH on a blood specimen to confirm the diagnosis.
2. At 3, 6, 9, and 12 months after initiating therapy, measure qPCR for *BCR-ABL1* transcripts. (If qPCR using the International Standard is not available, perform marrow cytogenetics.) If there is a rising level of *BCR-ABL1* transcript or 1 log increase after MMR achieved, qPCR should be repeated in 1 to 3 months.
3. At 12 months obtain marrow cytogenetics for cells with Ph chromosome if no CCyR or MMR.
4. Once CCyR is obtained, monitor qPCR on blood cells every 3 months for 3 years and then every 4 to 6 months, thereafter. If there is a rising level of *BCR/ABL1* transcripts (1 log increase after MMR achieved), repeat quantitative PCR in 1 to 2 months for confirmation.
5. These guidelines presume continued response to a TKI until CCyR achieved. If this does not occur see text for approach.
6. Mutation analysis should be performed with loss of chronic phase, loss of any previous level of response, inadequate initial response (*BCR/ABL1* transcripts > 10%) at 3 or 6 months or no CCyR at 12 or 18 months, and a 1-log increase in BCR/ABL after MMR once achieved.

CCyR, complete cytogenetic response; FISH, fluorescence in situ hybridization; MMR, major molecular response; Ph, Philadelphia; qPCR, quantitative polymerase chain reaction; TKI, tyrosine kinase inhibitor.
Data from http://www.nccn.org/professionals/physicians_gls/PDF/cml.pdf.

TABLE 46–5 MILESTONES FOR ASSESSING RESPONSE TO TYROSINE KINASE INHIBITORS

		Disease Response	
Time of Observation (months)	**Unsatisfactory**	**Suboptimal Response/Warning**	**Optimal Response**
3	No CHR and/or Ph+ > 95%	*BCR/ABL1* > 10% and/or Ph+ 36%–95%	*BCR/ABL1* ≤ 10% and/or MCyR
6	*BCR/ABL1* > 10% and/or no MCyR	*BCR/ABL1* 1%–10% and/or MCyR	*BCR/ABL1* < 1% and/or CCyR
12	*BCR/ABL1* > 1% and/or no CCyR	*BCR/ABL1* < 0.1%–1%	*BCR/ABL1* < 0.1%
18	No CCyR	CCyR if no MMR	CCyR or MMR

CCyR, complete cytogenetic response; CHR, complete hematologic response; MCyR, major cytogenetic response; MMR, major molecular response.
These data were derived from studies with imatinib but are applicable to therapy with any tyrosine kinase inhibitor (TKI) as initial therapy in chronic phase. "Unsatisfactory" implies the need to consider change in treatment approach, as appropriate for that patient. Usually this change is an increase in the dose of imatinib, a shift to an alternative TKI, or allogeneic hematopoietic cell transplantation, if eligible. These guidelines are approximate in that a patient showing continued response to a TKI can be continued on that therapy until a response plateau has been reached, at which time the response can be evaluated using the milestones described. The suboptimal category indicates at least closer monitoring is recommended. See text for further details.
Source: *Williams Hematology*, 9th ed, Chap. 89, Table 89–5.

Other Drugs

- Under special circumstances, several other second-line drugs can be considered: interferon-α, homoharringtonine, cytarabine, busulfan, and others. These are discussed in *Williams Hematology*, 9th ed, Chap. 89.

Allogeneic Hematopoietic Cell Transplantation

- Transplantation has decreased markedly in frequency in chronic phase CML because of the very favorable prognosis of those treated with tyrosine kinase inhibitors who achieve a complete cytogenetic remission.

— It should be considered in patients (1) who are eligible after all signs of continued improvement on TKIs have stopped and a complete cytogenetic remission has not been achieved, (2) who are intolerant to TKIs, or (3) who show progression of disease after using several TKIs.
— Patients younger than 70 years of age with an identical twin, a histocompatible sibling, or a histocompatible unrelated donor (matched using molecular methods) can be transplanted after intensive cyclophosphamide and fractionated total body radiation or a combination of busulfan and cyclophosphamide.
— Older patients may be transplanted with reduced-intensity conditioning regimens.
— Engraftment and 5-year survival can be achieved in approximately 60% of patients in chronic phase, and some patients are cured. In patients older than 50 years of age, the 5-year survival is somewhat decreased.
— Some patients die of severe graft-versus-host disease and opportunistic infection in the first 5 years after transplant.
— There is a 20% chance of relapse of CML in the 6 years after apparently successful transplant.
— Donor T lymphocytes play an important role in successful suppression of the leukemia by initiating a graft-versus-leukemia reaction.
— Disease status can be monitored by FISH (*BCR-ABL1* fusion) or RT-PCR (*BCR-ABL1* mRNA transcripts).
— Donor lymphocyte infusion (DLI) can produce remission in transplanted patients who have relapse of their disease. Ten million mononuclear cells/kg body weight may be sufficient to induce a graft-versus-leukemia effect and a remission. Response rates to this treatment may be as high as 80% and can be durable. Graft-versus-host disease and severe myelosuppression are the principal toxic risks.
— Post-transplant TKI therapy can be useful for patients who relapse, in patients in whom DLI does not provide benefit, and in patients felt to be at high-risk of relapse after transplantation because of advanced disease at time of transplant.

Radiation or Splenectomy

- Patients with marked splenomegaly and splenic pain or encroachment on the gastrointestinal tract who do not respond to drug therapy may benefit transiently from palliative splenic radiation. Marked splenomegaly is usually associated with acute transformation of the disease.
- Patients with focal extramedullary myeloid sarcomas with pain may benefit from local radiation.
- Splenectomy is of limited value but may be helpful in some patients, such as those with thrombocytopenia and massive splenomegaly, refractory to therapy. Postoperative complications are frequent.

COURSE AND PROGNOSIS

- The median survival has been greatly prolonged with tyrosine kinase inhibitors.
- As many as 90% of patients tolerant to TKIs may achieve a complete cytogenetic remission

after 5 years of treatment.

- In a major study, the 7-year overall survival was 86% in those able to be maintained on imatinib. This finding may be improved with the use of second-generation TKIs, nilotinib and dasatinib.
- **Table 46–6** provides the relative 5-year survival by age at diagnosis.
- Patients in CMR treated with imatinib with undetectable BCR-ABL1 transcripts (more than five log reduction) in whom imatinib therapy was stopped indicated that some may have enduring remissions off TKI treatment. Importantly, those that relapsed were successfully retreated with imatinib.

TABLE 46–6 CHRONIC MYELOGENOUS LEUKEMIA: 5-YEAR PERIOD RELATIVE SURVIVAL RATES (2004-2012) BY AGE AT DIAGNOSIS

Age (years)	Percent of Patients*
< 45	86
45–54	82
55–64	70
65–74	51
> 75	27

*Percent rounded to nearest whole number.

Data from Surveillance, Epidemiology, End Results Cancer Statistics: *5-Year Survival Rates, Table 13.6, All Races and Sexes*. National Cancer Institute, Washington, DC. Available at http://www.seer.cancer.gov.

ACCELERATED PHASE OF CML

- The natural history of CML is for patients to enter a more aggressive phase of disease characterized by severe dyshematopoiesis, increasing splenomegaly, extramedullary tumors, and, often, development of a clinical picture of acute leukemia—the "blast crisis."
- Although this evolution occurs in patients who are resistant or intolerant to TKIs, the frequency per unit time has decreased markedly as a result of the prolonged remissions induced by these agents. It may take another decade to determine the rate of this event in persons who enter a complete cytogenetic remission after treatment with a TKI and whether the nature of the accelerated phase mimics the one we have observed over the past 150 years.
- The Ph chromosome and *BCR-ABL1* oncogene persist in myeloid or lymphoid blasts in the accelerated phase, but additional chromosomal abnormalities often develop, such as trisomy 8, trisomy 19, isochromosome 17, or gain of a second Ph chromosome, and characteristic molecular genetic changes have been identified.

Clinical Features

- Unexplained fever, night sweats, weight loss, malaise, and arthralgias occur.
- New extramedullary sites of disease containing Ph chromosome-positive blast cells may develop.
- There is a diminished responsiveness to previously effective drug therapy.

Laboratory Findings

Blood Findings

- There is progressive anemia with increasing anisocytosis and poikilocytosis and increased numbers of nucleated red cells.
- An increasing proportion of blasts in the blood or marrow may reach 50% to 90% at the time of blastic crisis.
- The percentage of basophils increases (occasionally to levels of 30%–80%).
- Hyposegmented neutrophils appear (acquired Pelger-Huët abnormality).
- Thrombocytopenia occurs.

Marrow Findings

- Marked dysmorphic changes may be seen in any or all cell lineages, or florid blastic transformation may occur.

Blast Crisis

- Extramedullary blast crisis is the first sign of the accelerated phase in about 10% of patients. Lymph nodes, serosal surfaces, skin, breast, gastrointestinal or genitourinary tracts, bone, and the central nervous system are most often involved.
- Central nervous system involvement is usually meningeal. Symptoms and signs are headache, vomiting, stupor, cranial nerve palsies, and papilledema. The spinal fluid contains leukemic cells, including blasts, and the protein level is elevated.
- Acute leukemia develops in most patients in the accelerated phase. It is usually myeloblastic or myelomonocytic but may be of any cell type.
- About one-third of patients develop acute lymphoblastic leukemia (ALL), with blast cells that contain terminal deoxynucleotidyl transferase, an enzyme characteristic of ALL, and surface markers typical of B cells.

Treatment of Patients with AML Phenotype

- Acute myelogenous leukemic transformation (any subtype can occur) is essentially incurable with chemotherapy presently available.
- The strategy that is most likely to result in a prolonged remission is allogeneic hematopoietic cell transplantation, albeit with a low long-term remission rate. Thus, drug therapy, in patients eligible for transplantation, is focused on a remission sufficiently long to accomplish transplantation in that state.
- One can start with imatinib, 600 mg/d; dasatinib, 140 mg/d or 70 mg q12h; or nilotinib, 400 mg q12h. Then one should use the alternative drug if the response is inadequate. Good remissions with one but not another TKI have been observed. Remission or reversion to chronic phase usually is short-lived, and allogeneic hematopoietic cell transplantation from a histocompatible donor should be urgently considered for eligible patients.
- TKI can be combined with mitoxantrone plus etoposide or cytarabine (an AML drug protocol) depending on patient's ability to tolerate such an approach (see Chap. 45).

Treatment of Patients with Acute Lymphoblastic Leukemia Phenotype

- Start with dasatinib, 140 mg/d, or 70 mg q12h or nilotinib, 400 mg q12h. These TKIs can induce a remission in patients with an ALL phenotype. If remission ensues, consider allogeneic stem cell transplantation if patient is eligible and a donor is available.
- If relapse occurs after a TKI, consider ALL drug protocol such as vincristine sulfate, 1.4 mg/m^2 (to a maximum of 2 mg/dose) intravenously once per week, and prednisone, 60 mg/m^2 per day orally. A minimum of 2 weeks of therapy should be given to judge responsiveness (see Chap. 54).
- About one-third of patients will reenter the chronic phase with this treatment, but the median duration of remission is about 4 months, and relapse should be expected. Because acute lymphocytic blast crisis occurs in about 30% of cases, a 30% response represents about 9% of all patients entering ALL blast crisis.
- The response rate may be improved by more intensive therapy, similar to that used in de novo ALL, but not dramatically so. Most patients do not respond to repeat therapy.
- Allogeneic hematopoietic cell transplantation from a histocompatible donor may lead to prolonged remission in blast crisis. Thus, eligible patients who enter remission with therapy should be strongly considered for transplantation.

ACUTE LEUKEMIAS WITH THE PHILADELPHIA CHROMOSOME

- Ph chromosome-positive AML appears, in some cases, to be instances of CML, presenting in myeloid blast crisis, while other cases appear to be unrelated to CML.
- Ph chromosome-positive ALL represents about 3% of cases of childhood ALL and 20% of cases of adult ALL.
- In both adults and children, the prognosis is worse for those patients with leukemic cells containing the Ph chromosome.
- Some Ph chromosome-positive ALL patients have the same size BCR-ABL1 fusion oncoprotein (p210) that characterize CML and are believed to be patients with CML presenting in lymphoid blast crisis. The remaining patients have a p190 fusion protein and are thought to have de novo ALL.
- Treatment generally combines a TKI, as used for CML blast crisis, with intensive therapy for AML or ALL, as described in Chaps. 45 and 54, respectively.

CHRONIC MYELOID LEUKEMIAS WITHOUT THE PHILADELPHIA CHROMOSOME (TABLE 46–7)

TABLE 46–7 TYPES OF CHRONIC MYELOGENOUS LEUKEMIA

Type of Chronic Myelogenous Leukemia	Molecular Genetics	Major Clinical Features
BCR rearrangement-positive chronic myelogenous leukemia	> 95% p210BCR-ABL1; < 5% p190 or p230	Splenomegaly in 80% of cases; WBC > 25 × 10^9/L; blood blasts < 5%; absolute basophilia in virtually all cases. Ph chromosome in 90% of cases; *BCR* gene rearrangement in 100% of cases

Chronic myelomonocytic leukemia	> 40% mutation in *SRSF2* gene and 90% have a mutation in one of nine genes. Various cytogenetic abnormalities	Anemia, monocytosis > 1.0×10^9/L; blood blasts < 10%; increased plasma and urine lysozyme; *BCR-ABL1* absent; rare cases with *PDGFR*-β mutation respond to imatinib
Chronic eosinophilic leukemia	Various cytogenetic abnormalities; *PDGFR*-α mutations in some cases	Blood eosinophil count > 1.5×10^9/L; cardiac and neurologic manifestations common; a proportion of cases have *PDGFR-α* mutations and are responsive to imatinib mesylate
Chronic basophilic leukemia	Various cytogenetic abnormalities	Only five cases reported; hemoglobin 6–13 g/dL; basophilia of 3.4–41 × 10^9/L; two of five cases with splenomegaly; very cellular marrow (> 90%) in each case with mild increase in type III collagen, and megakaryocytic dysmorphia; increase in marrow mast cells in three of five cases
Juvenile myelomonocytic leukemia	*RAS* pathway mutations (*PTPN11*, *NF1*, *NRAS*, *KRAS* and *CBL*) in ~90% of cases. Various cytogenetic changes	Infants and children < 4 years; eczematoid or maculopapular rash; anemia and thrombocytopenia; increased Hgb F in 70% of cases; neurofibromatosis in 10% of cases; abnormality of chromosome 7 (eg, del 7, del 7q, etc.) in approximately 20% of patients; *BCR* rearrangement absent
Chronic neutrophilic leukemia	Colony-stimulating factor 3 receptor gene *(CSF3R)* alone (~30% of cases); a combination of mutated *CSF3R* and a SET-binding protein gene *(SETBP1)* mutation (~60% of cases); the *JAK2*V617F mutation alone (~10% of cases)	Segmented neutrophilia > 20×10^9/L; splenomegaly >90% of cases; no blood blasts; platelets > 100×10^9/L; 75% of cases have normal cytogenetics; *BCR* rearrangement absent
BCR rearrangement–negative chronic myelogenous leukemia	Various cytogenetic changes	Clinical findings indistinguishable from *BCR* rearrangement-positive CML; Ph chromosome and *BCR-ABL1* fusion gene absent
Atypical myeloproliferative disease	Various cytogenetic changes	Usually older patient (> 65 years); variable blood cell changes: anemia, granulocytosis, normal or decreased platelet counts; hypercellular marrow, marrow blasts < 10%; dysmorphia of blood and marrow cells common (eg, Pelger-Huët neutrophils, dyserythropoiesis, and megakaryopoiesis; often splenomegaly)

Hgb, hemoglobin; PDGFR, platelet-derived growth factor receptor; Ph, Philadelphia; WBC, white blood cells.
Source: *Williams Hematology*, 9th ed, Chap. 89, Table 89–9.

Chronic Myelomonocytic Leukemia

- Approximately 75% of patients are older than 60 years of age at onset.
- The male-to-female ratio is 1.4:1.
- Onset is insidious, with weakness, exaggerated bleeding, fever, and infection most common.
- Hepatomegaly and splenomegaly occur in about 50% of cases.
- Usually anemia and monocytosis (≥ 1.0×10^9/L) are present; less than 10% of cells in blood are myeloblasts.
- White cell count may be decreased, normal, or elevated and can reach levels of more than 200×10^9/L.
- Marrow is hypercellular, with granulomonocytic hyperplasia.
- Myeloblasts and promyelocytes are increased but are less than 30% of cells in marrow.
- Approximately one-third of patients have an overt cytogenetic abnormality (eg, monosomy 7, trisomy 8, complex karyotype).
- Plasma and urine lysozyme concentrations are nearly always elevated.
- Thirty-five percent of patients have *N-RAS* or *K-RAS* gene mutations.

- Thirty percent to 50% of patients have a *SRSF2* mutation. The latter mutation frequently coincident with *TET2* or *EZH2* mutations.
- Other gene mutations include *RUNX1, IDH1/2, CBL, JAK2, TET2, EZH2,* and several more.
- The Ph chromosome and *BCR-ABL1* are absent.
- More than 90% of patients have an identifiable mutated gene.
- A small percentage of patients have a translocation fusing the *PDGFR*-α gene with one of several gene partners (eg, *TEL*). These cases may be associated with prominent eosinophilia.
- Several prognostic variables have been identified. Among the most compelling are height of blast cell count, severity of anemia, serum LDH, and spleen size at time of diagnosis.
- Median survival is approximately 12 months, with a range of 1 to 60.
- Approximately 20% of patients progress to overt AML.

Treatment

- No standard or highly successful therapy has been developed. See *Williams Hematology,* 9th ed, Chap. 89.
- Low-dose cytosine arabinoside, hydroxyurea, etoposide may induce occasional partial remissions for short periods of time, measured in months usually.
- 5-Azacytidine or decitabine has resulted in improvement in a minority of patients.
- Occasional patients with *PDGFR-partner* fusion oncogenes can respond to imatinib, 400 mg/d, orally or an alternative TKI. A TKI may result in a hematological, cytogenetic, and molecular remission.
- Other cytotoxic drugs, maturation-enhancing agents, interferons, and growth factors have been used.
- Marrow transplantation has yielded more favorable results than other therapies but the afflicted population has a median age of 70 years, making transplantation for most problematic.

Chronic Eosinophilic Leukemia

- This *BCR-ABL1*–negative chronic clonal myeloid disease is manifested by prolonged exaggerated blood eosinophilia unexplained by parasitic or allergic disease.
- Fever, cough, weakness, easy fatigability, abdominal pain, maculopapular rash, cardiac symptoms, signs of heart failure, and a variety of neurologic symptoms may coexist in some combination.
- Eosinophilia is a constant finding. Anemia is often present. Platelet counts are normal or mildly decreased. Total white cell counts are normal or elevated in relationship to the degree of eosinophilia, which may be as high as 100×10^9/L.
- Marrow examination reveals markedly increased eosinophilic myelocytes and segmented eosinophils. Reticulin fibrosis may be present. Occasional Charcot-Leyden crystals may be found. In patients with the *FIP1L1-PDGFR*-α mutation, spindle-shaped (neoplastic) mast cell aggregates are invariably present.
- Many different cytogenetic abnormalities can be found. Notably a high frequency of translocations involving chromosome 5 occur; for example, t(1;5) t(2;5), t(5;12), t(6;11), 8p11, trisomy 8, and infrequently others can be present. *PDGFR*-α, present on chromosome 5, is sometimes involved in the translocation.

- Immunophenotyping and PCR do not show evidence of clonal T-cell population or T-cell receptor rearrangement.
- Pulmonary function studies may be consistent with fibrotic lung disease.
- Echocardiography may detect mural thrombi, thickened ventricular walls, and valvular dysfunctions.
- Skin, neural, and brain biopsy, if indicated, shows intense eosinophilic infiltrates.
- A subset of patients with elevated serum tryptase (> 11.5 ng/mL), intensely hypercellular marrow eosinophilia with a high proportion of immature eosinophils, high serum B_{12} and IgE levels, more prone to pulmonary and endocardial fibrosis, and the *FIL1L1-PDGFR*-α oncogene, are responsive to TKIs (eg, imatinib).
  — This mutation can be found in approximately 15% of patients with eosinophilia unrelated to parasitic or allergic diseases. This mutation may be responsive to imatinib mesylate or its congeners.
- Patients who are unresponsive to imatinib mesylate or its second-generation congeners, should be considered for allogeneic hematopoietic cell transplantation, if eligible, and with an appropriate donor.
- Other patients may be treated with glucocorticoids, hydroxyurea, or anti-IL-5 antibodies in an effort to decrease the eosinophil count and ameliorate the eosinophil-induced deleterious tissue effects.
- If the patient is not imatinib-sensitive or eligible for transplantation, chronic eosinophilic leukemia is difficult to control. Occasional patients may progress to acute eosinophilic or myelogenous leukemia. Cardiac, pulmonary, and neurologic manifestations, if not stabilized, contribute to morbidity and mortality.

Juvenile Chronic Myelomonocytic Leukemia

- This occurs most often in infants and children younger than 4 years of age.
- Twenty percent of patients have *RAS* mutations.
- Ten percent have type 1 neurofibromatosis (400 times the expected frequency) and *NF1* mutations.
- Somatic mutation in the *PTPN11* gene occurs in one-third of affected children and chromosome abnormalities such as -7, del(7q) occur in approximately 20% of patients.
- The disease appears to be related, in part, to an increase in sensitivity of myeloid cells to the proliferative effects of granulocyte-monocyte colony-stimulating factor.
- Infants have failure to thrive. Children present with fever; malaise; persistent infection; and skin, oral, or nasal bleeding.
- Fifty percent of patients have eczematoid or maculopapular skin lesions.
- Café-au-lait spots (neurofibromatosis type 1) may occur.
- Nearly all patients have splenomegaly, sometimes massive.
- Anemia, thrombocytopenia, and leukocytosis are common.
- The blood contains monocytosis of 1.0 to 100 × 10^9/L, with immature granulocytes, including a low percentage of blast cells, and nucleated red cells.
- Fetal hemoglobin concentration is increased in about two-thirds of the patients.
- The marrow is hypercellular as a reflection of granulocytic hyperplasia, with an increase in

monocytes and leukemic blast cells. Erythroblasts and megakaryocytes are decreased.
- The disease is refractory to chemotherapy. Four to six drug combinations (eg, cytarabine, etoposide, vincristine, and isotretinoin) have induced partial remissions in some patients and may extend survival, although median survival has been less than 30 months.
- Allogeneic hematopoietic cell transplantation can prolong survival, but cures, even with this approach, are difficult to achieve.
- A rapid search for a suitable donor (matched-related, matched-unrelated) is important.
- Allogeneic hematopoietic cell transplantation can achieve 5-year survivals of up to 50% in children up to 1 year of age and approximately 30% in children older than 1 year of age.
- Monosomy 7 is a poor prognostic indicator of transplantation results.
- The median survival is less than 2 years, but occasional patients (usually infants) or massively treated patients survive for longer periods with the disease.
- A minority of children have a smoldering course and survive for approximately 2 to 4 years, with rare exceptions for as long as a decade.
- The disease may undergo clonal evolution to acute leukemia, usually myelogenous type.

Chronic Neutrophilic Leukemia

- A leukocyte count of 25 to 50 × 10^9/L, with about 90% to 95% mature neutrophil is characteristic.
- Neutrophil alkaline phosphatase activity is increased.
- Serum vitamin B_{12} and vitamin B_{12}-binding protein are markedly increased.
- Marrow shows granulocytic hyperplasia usually with a very low proportion of blasts (1%–3%). Rarely, dysmorphic features of neutrophils are evident (acquired Pelger-Hüet nuclear shape).
- Ph chromosome and *BCR* gene rearrangement are absent.
- Approximately 25% of patients have random clonal cytogenetic abnormality (eg, trisomy 9 or 21 or del(20q)).
- *CSF3R* and *SETBP1* gene mutations, either, alone or combined, occur in 60% of patients. A *JAK2*^{V617F} mutation is present in about 10% of patients.
- Liver and spleen are enlarged and are infiltrated with immature myeloid cells and megakaryocytes.
- A concordance with essential monoclonal gammopathy or myeloma has been noted.
- The neoplasm is rare, and no systematic studies of treatment are available.
- Hydroxyurea and cytarabine have been used with transient benefit.
- An inhibitor of JAK2 (eg, ruxolitinib) may be useful in cases with either a *JAK2* mutation or a *CSF3R*^{T618I} mutation, and a TKI (eg, dasatinib) may be useful in those with a *CSF3R*^{S783Fs} mutation.
- Allogeneic hematopoietic cell transplantation may be curative.
- The condition may terminate in typical AML.
- Survival is usually 0.5 to 6.0 years (median 2.5 years).

Philadelphia Chromosome–Negative CML

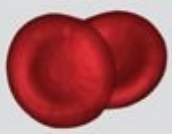
- About 4% of patients with a phenotype indistinguishable from *BCR-ABL1*–positive CML do

not have the Ph chromosome or *BCR* gene rearrangement on assiduous search.

- Median age is 66 years and ranges from 25 to 90 years.
- Median white cell count is lower (approximately 40×10^9/L), but the range is wide (11 to 296 $\times 10^9$/L).
- The morphologic features in blood and marrow are characteristic of classic CML.
- Splenomegaly in only 50% at diagnosis.
- Disease progression marked by cytopenias. Median survival is 2 years, and only 7% survive for more than 5 years.
- One-third of patients monitored until death developed AML.
- Occasional patients have extended complete responses to interferon-α. Hydroxyurea is often used to control the white cell count or decrease splenomegaly (palliative therapy).

Atypical Myeloproliferative Disease (Atypical CML)

- This disorder usually occurs in older patients (60 to 90 years).
- It does not meet the criteria for a classical myeloproliferative disease (eg, CML, CMML, polycythemia vera, essential thrombocythemia, myelodysplastic syndrome).
- It does not have classical translocation such as that involving *BCR*, *PDGFR*α, and so on.
- Hepatomegaly or splenomegaly are infrequent.
- Serum LDH may be elevated.
- Anemia and granulocytosis are very common, including a low proportion of immature granulocytes, but with a very low blast count (< 5.0%). Monocytes are not increased.
- Marrow is usually hypercellular with a low blast count (typically < 10%).
- Neutrophilic dysmorphia may be present (eg, acquired Pelger-Hüet anomaly).
- Clonal cytogenetic abnormalities, if present, are random (eg, trisomy 8, del(20q); assorted others).
- There is no specific therapy. Azacytidine or hydroxyurea (if hyperproliferative) may be used with variable results. Red cell and platelet transfusion are necessary as required.
- In a suitable patient with an appropriate donor, allogeneic hematopoietic cell transplantation can be considered (nonmyeloablative or ablative).
- Median survival is approximately 18 months, but longer survival with palliative therapy can occur.
- Clonal evolution to acute myelogenous leukemia can occur.



For a more detailed discussion, see Jane L. Liesveld and Marshall A. Lichtman: Chronic Myelogenous Leukemias and Related Disorders, Chap. 98 in *Williams Hematology*, 9th ed.

CHAPTER 47

Primary Myelofibrosis

DEFINITION

- Primary myelofibrosis is a chronic myeloid neoplasm that originates in mutations in a multipotential hematopoietic cell, possibly the lymphohematopoietic stem cell. The disease is characterized by (1) anemia; (2) splenomegaly; (3) increased CD34+ cells, immature granulocytes, erythroid precursors, and teardrop-shaped red cells in the blood; (4) increased dysmorphic megakaryocytes, the cytokines from which induce marrow fibrosis; and (5) osteosclerosis.

EPIDEMIOLOGY

- Onset is characteristically after age 50 years.
- Median age at diagnosis is approximately 70 years.
- Adult males and females are affected equally.
- Incidence is approximately 1.0 case per 100,000 in persons of European descent.

PATHOGENESIS

- Origin is in the neoplastic transformation of a multipotential primitive hematopoietic cell.
- In approximately 50% of patients, a mutation, *JAK2* V617F, in approximately 35% of patients a mutation, *CALR*, and in approximately 5% of patients a mutation, *MPL*, can be found in the blood cells. Approximately 10% of patients' blood cells do not have one of these mutations.
- Frequently other mutations may be found in patients' blood cells, including, for example, those involving *TET2*, *ASXL1*, *DNMT3A*, *EZH2*, *IDH1*, *TP53*, and *CBL*.
- Constitutive mobilization and circulation of CD34+ cells occurs as a result of epigenetic methylation of the CXCR4 promoter, leading to decreased expression of CXCR4 on CD34+ cells and their enhanced migration from marrow to blood.
- CD34+ cells in this disorder generate about 24-fold the megakaryocytes in culture than do CD34+ cells from normal persons.

Fibroplasia

- Reticulin fibers (type III collagen), as detected by silver staining, are increased in the marrow in most patients. Fibrosis may progress to thick collagen bands (type I collagen) identified with the trichrome stain.
- Increased plasma concentrations of procollagen III amino-terminal peptide, prolylhydroxylase,

and fibronectin are present.

- The extent of fibrosis is correlated with the prevalence of dysmorphic megakaryocytes and release of fibroblast growth factors from the megakaryocyte α granules (eg, platelet-derived growth factor, basic fibroblast growth factor, epidermal growth factor, transforming growth factor-β, and others).
- The fibroblastic proliferation in the marrow is a reaction to the cytokines released by an increased density of dysmorphic megakaryocytes, not an intrinsic part of the clonal expansion of hematopoietic cells.
- Thicker bands of collagen fibrosis (type I collagen) may develop as the marrow fibrosis advances.

CLINICAL FEATURES

- The median age at diagnosis is approximately 70 years, but the disorder may occur at any age.
- The sex incidence is equal in adults, but the disorder occurs in twice as many females as males in young children.
- Rarely, myelofibrosis is preceded by extended high-dose ionizing radiation.
- Approximately 25% of patients are asymptomatic at time of diagnosis.
- Fatigue, weakness, shortness of breath, palpitations, weight loss, night sweats, and bone pain are common presenting symptoms.
- Wasting, peripheral edema, or bone tenderness may occur.
- Left upper quadrant fullness, pain or dragging sensation, left shoulder pain, and early satiety may result from splenic enlargement and/or infarction.
- Splenomegaly is present in virtually all patients at the time of diagnosis and is massive in one-third of cases.
- Hepatomegaly is present in two-thirds of patients.
- Neutrophilic dermatosis (Sweet syndrome) may occur.

SPECIAL CLINICAL FEATURES

Prefibrotic Stage

- Many experts believe that one can identify a prefibrotic stage of myelofibrosis in which early changes, such as slight anemia, slight neutrophilia, and thrombocytosis occur. The latter is a constant finding. The marrow biopsy may not show increased reticulin fibers at this stage. A constant feature, however, is an increase in marrow megakaryocytes, often with dysmorphic features. Large and small megakaryocytes are admixed. Use of a megakaryocyte marker, such as CD61, to stain the marrow, makes the increased megakaryocyte population evident.
- The presence of a mutation in the *JAK2*, *CALR*, or *MPL* gene would be strong additional evidence of prefibrotic myelofibrosis, although in the case of a *JAK2* mutation and the hematological findings described, essential thrombocythemia should be considered unless and until the other classical findings of myelofibrosis appear. See **Table 47–1**.

TABLE 47–1 DIAGNOSTIC FINDINGS IN PRIMARY MYELOFIBROSIS

PREFIBROTIC STAGE
Anemia may be absent or mild
Leukocytosis may be absent or slight
Thrombocythemia is invariable
BCR-ABL fusion gene absent
Presence of *JAK2, CALR, or MPL* mutations indicative of diagnosis of myeloproliferative disease (one of these mutations present in ~90% of patients)
Cellular marrow with mild increase in granulopoiesis; increased megakaryocytes, clusters of very dysmorphic megakaryocytes and megakaryocytic nuclei; no to very slight increase in reticular fibers on silver stain
Palpable splenomegaly infrequent
Absent or slight anisopoikilocytosis including teardrop red cells
FULLY DEVELOPED STAGE
Marrow reticulin fibrosis plus or minus collagen fibrosis
BCR-ABL fusion gene absent
JAK2, CALR, or MPL mutation in approximately 90% of patients
Splenomegaly
Anisopoikilocytosis with teardrop red cells in virtually every oil immersion field
Immature myeloid cells in blood
Increased CD34+ cells in blood
Nucleated red cells in blood
Marrow usually hypercellular but invariably has increased megakaryocytes, clusters of highly dysmorphic megakaryocytes, and megakaryocyte bare nuclei regardless of overall marrow cellularity

Source: *Williams Hematology*, 9th ed, Chap 86, Table 86–2.

Extramedullary Hematopoiesis and Fibrohematopoietic Tumors

- Presence in liver and, especially, spleen, in this disorder contributes to the organomegaly.
- Extramedullary hematopoiesis is not effective as a source of blood cell production.
- Hematopoietic foci are often present in adrenals, kidneys, lymph nodes, bowel, breast, lungs, and other sites.
- Hepatic extramedullary hematopoiesis worsens after splenectomy and leads to an enlarging liver, sometimes massive, and can result in hepatic failure.
- Identification of a mass on imaging, unexpected neurologic signs, or other unexpected findings should raise the consideration of a fibrohematopoietic tumor that can arise in any tissue or organ.
- Central nervous system sites of extramedullary hematopoiesis can be associated with subdural hemorrhage, delirium, increased cerebrospinal fluid pressure, papilledema, coma, motor and sensory impairment, and paralysis.
- Hematopoietic foci on serosal surfaces can cause effusions in the thorax, abdomen, or pericardial spaces.

Portal Hypertension and Esophageal Varices

- Strikingly increased splenoportal blood flow and decreased hepatic vascular compliance can lead to portal hypertension, ascites, esophageal and gastric varices, intraluminal gastrointestinal bleeding, and hepatic encephalopathy.
- The hepatic venous pressure gradient, normally less than 6 torr, is markedly elevated.
- Portal vein thrombosis may occur.

Pulmonary Artery Hypertension

- About one-third of patients with primary myelofibrosis have an elevated pulmonary artery pressure, the fraction that is symptomatic is relatively small.

Bone Changes

- Osteosclerosis may develop with increased bone density evident on radiographs and marrow biopsy.
- Periostitis may lead to severe bone pain.
- Increased bone blood flow (up to 25% of cardiac output) may contribute to the development or accentuation of congestive heart failure.

Immune Findings

- Various immune products may develop, such as anti–red cell, antineutrophil, antiplatelet antibodies, antinuclear antibodies, or antiphospholipid antibodies.
- Inflammatory cytokines, including interleukin(IL)-1β, IL-6, IL-8, and tumor necrosis factor-α, are frequently elevated and may contribute to constitutional symptoms.

Thrombotic Arterial or Venous Events

- Incidence is elevated but not to the extent seen in polycythemia vera (see Chap. 41). Age, elevated platelet count, and coincident vascular disease are the three principal risk factors for thrombosis.
- Portal (or other mesenteric) vein thrombosis may be presenting event in some patients.

LABORATORY FEATURES

Blood Findings

- Normocytic-normochromic anemia is found in most patients (mean hemoglobin = 105 g/L with a very wide range).
- Anisopoikilocytosis, and teardrop red cells (dacryocytes) are constant findings (**Figure 47–1A**).
- Occasional nucleated red cells are seen in the blood film in most patients.
- Anemia may be worsened by an expanded plasma volume and/or splenic trapping of red cells.
- Reticulocyte count is variable but usually the absolute reticulocyte count is low for the degree of anemia.
- Hemolysis may be present and rarely may be autoimmune, with a positive antiglobulin (Coombs) test.
- Acquired hemoglobin H disease with hypochromic-microcytic and teardrop red cells and red cell inclusions (hemoglobin H precipitates) stained by brilliant cresyl blue can occur rarely, admixed with typical white cell and platelet changes of myelofibrosis.
- Erythroid aplasia may coexist, rarely.
- The total leukocyte count averages approximately 12×10^9/L in many studies and is usually less than 40×10^9/L, but may be as high as 200×10^9/L with neutrophilia predominating.
- Neutropenia occurs in 15% of patients at the time of diagnosis.

- Myelocytes and metamyelocytes are present in the blood of all patients, along with a low proportion of blast cells (0.5%–5%).
- Neutrophils may have impaired phagocytosis, decreased myeloperoxidase activity, and other functional abnormalities.
- Basophils may be slightly increased in number.
- About 40% of patients have elevated platelet counts, and 33% have mild to moderate thrombocytopenia.
- Giant platelets, abnormal platelet granulation, and occasional circulating micromegakaryocytes are characteristic.
- The closure time may be prolonged and platelet aggregation with epinephrine may be impaired along with depletion of dense granule ADP and decreased lipoxygenase activity in platelets.
- Pancytopenia occurs in 10% of patients at presentation, usually secondary to ineffective hematopoiesis coupled with splenic sequestration.
- The variable blood counts are a reflection of an unpredictable combination of effective or ineffective hematopoiesis, expanded hematopoiesis in one or another blood cell lineage, exaggerated late precursor apoptosis in one or another lineage, effect of splenomegaly on blood cell pooling or shortened survival, presence of anti–blood cell antibodies, and other factors. Thus, (1) anemia, neutrophilia, and thrombocytosis; (2) anemia, neutropenia, and thrombocytosis; (3) anemia, neutrophilia, and thrombocytopenia; and (4) anemia, neutropenia, and thrombocytopenia may be present at diagnosis. The first pattern mentioned is the most common. Occasionally, anemia may not be present initially.
- Increased numbers of circulating multipotential, granulocytic, monocytic, and erythroid progenitor cells are found.
- Increased CD34+ cells in the blood are characteristic and more than 15×10^6/L is virtually diagnostic of primary myelofibrosis. Their frequency is roughly correlated with extent of disease and disease progression.
- Serum levels of uric acid, lactic acid dehydrogenase, alkaline phosphatase, and bilirubin are often elevated.
- Serum levels of albumin, cholesterol, and high-density lipoproteins are usually decreased.
- Immune manifestations include anti–red cell antibodies, antinuclear antibodies, anti–gamma globulins, antiphospholipid antibodies, and others may be found.

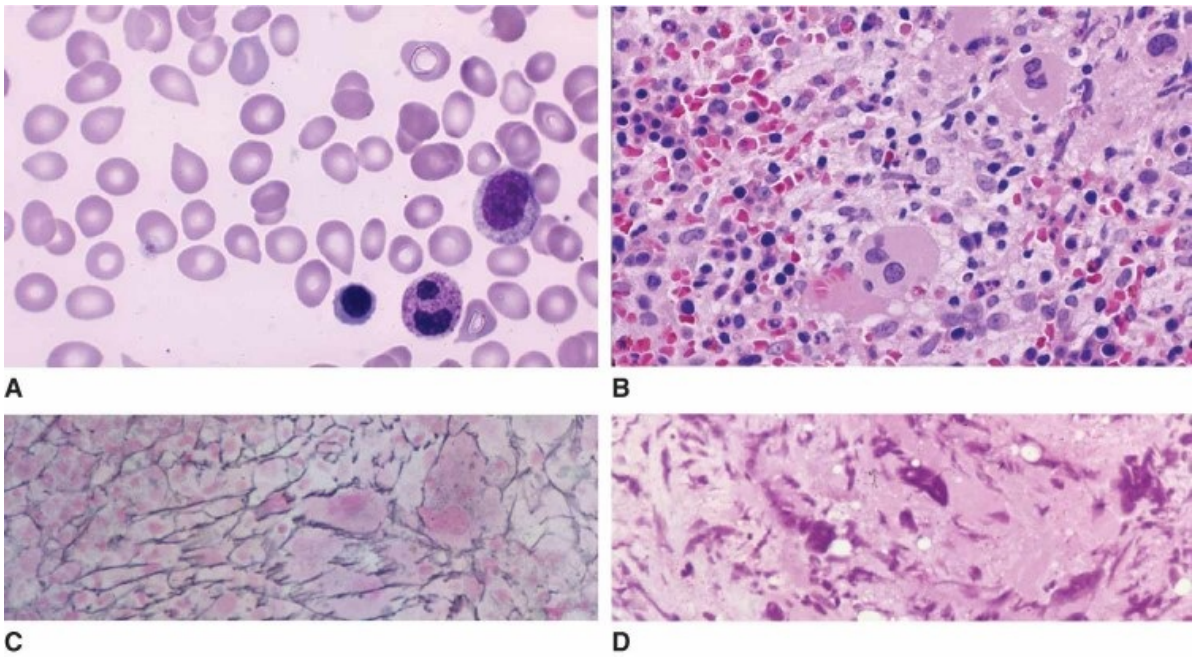


FIGURE 47–1 Blood film and marrow sections from patients with primary myelofibrosis. **A.** Blood film. Characteristic teardrop poikilocytes, a nucleated red cell, and a segmented neutrophil with a dysmorphic nucleus are evident. **B.** Marrow section. Low power. Hypercellular marrow with increased number of hypolobular megakaryocytes. **C.** Marrow section. Silver impregnation stain. Marked increase in argentophilic fibers representing collagen type III (reticulin). **D.** Marrow section. Collagen fibrosis with extensive replacement of marrow with swirls of collagen fibers. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

Marrow Findings

- See **Figure 47–1**.
- Marrow aspiration is often unsuccessful because of fibrosis ("dry tap").
- Biopsy is often cellular, with variable degrees of erythroid, granulocytic, and megakaryocytic expansion.
- Silver stain shows increased reticulin, often extreme. Gomori trichrome stain may show collagen fibrosis, which can be extreme, along with osteosclerotic changes.
- Megakaryocytes are prominent even in hypocellular, densely fibrotic specimens, and they are usually strikingly dysmorphic: giant or dwarf forms, abnormal nuclear lobulation, and naked nuclei.
- Increased marrow neoplastic megakaryocytopoiesis is the most constant and characteristic feature of the disease and accounts for several of the secondary manifestations (eg, marrow fibrosis).
- Granulocytes may have hyperlobulation or hypolobulation, acquired Pelger-Huët anomaly, nuclear blebs, and nuclear-cytoplasmic asynchrony.
- Small clusters of blast cells may be present.
- Dilated marrow sinusoids are common, with intrasinusoidal immature hematopoietic cells and megakaryocytes.
- Increased marrow microvessel density is present (enhanced angiogenesis) in 70% of patients.

CYTOGENETICS

- Cytogenetic abnormalities are present in approximately 50% of cases. Aneuploidy (monosomy

or trisomy) or pseudodiploidy (partial deletions and translocations) are common. These cytogenetic abnormalities are not seen in fibroblasts. Because marrow aspirates may be difficult to obtain because of the fibrosis, a panel of fluorescence in situ hybridization probes that reflect the most prevalent abnormalities seen may be used to search for cytogenetic abnormalities (see discussion in *Williams Hematology*, 9th ed, Chap. 86).

Magnetic Resonance Imaging

- Marrow fibrosis alters the T1-weighted images that normally result from marrow fat. As cellularity and fibrosis increase, hypointensity of T1- and T2-weighted images develop. Primary myelofibrosis cannot be distinguished from secondary, although the clinical and laboratory findings usually make the distinction evident. Osteosclerosis and vertebral changes (sandwich vertebrae) can be detected. Periosteal reactions also can be identified with this imaging technique.

DIFFERENTIAL DIAGNOSIS

- Chronic myelogenous leukemia. In this leukemia, the white count is often greater than 100×10^9/L at presentation, red cell shape is usually approximately normal, and marrow fibrosis is minimal. The Philadelphia chromosome and/or *BCR-ABL* fusion gene is present in hematopoietic and blood cells (see Chap. 46).
- Myelodysplasia. Pancytopenia and maturation abnormalities may occur in both myelodysplasia and myelofibrosis. Teardrop-shaped red-cell frequency (every oil immersion field), prominent splenomegaly, and marrow fibrosis should help distinguish primary myelofibrosis from a myelodysplastic syndrome in most cases (see Chap. 44).
- Hairy cell leukemia may show findings consistent with primary myelofibrosis (anemia, splenomegaly, marrow fibrosis), but the presence of abnormal mononuclear (hairy) cells in marrow and blood with characteristic CD phenotype separates the two disorders (see Chap. 56).
- Disorders with reactive marrow fibrosis include metastatic carcinoma (eg, breast, prostate), disseminated mycobacterial infection, mastocytosis, myeloma, renal osteodystrophy, angioimmunoblastic lymphadenopathy, gray platelet syndrome, systemic lupus erythematosus, polyarteritis nodosa, neuroblastoma, rickets, Langerhans cell histiocytosis, and malignant histiocytosis.
- Primary autoimmune myelofibrosis has no associated evidence of a connective tissue disease, especially systemic lupus erythematosus. Anemia and sometimes pancytopenia may be present, but neither the blood cell changes indicative of primary myelofibrosis (teardrop cells, anisopoikilocytosis) nor splenomegaly is present. It often responds to glucocorticoids.
- Disorders occurring coincidentally with myelofibrosis include lymphoma, chronic lymphocytic leukemia, hairy cell leukemia, macroglobulinemia, amyloidosis, myeloma, and monoclonal gammopathy, and virtually all cases of pulmonary hypertension.
- All clonal myeloid diseases may have increased reticulin in the marrow, but only primary myelofibrosis has collagen fibrosis.
- Acute megakaryocytic leukemia may show intense marrow fibrosis (acute myelofibrosis), but

the characteristic red cell changes and splenomegaly are absent and blast cells and very dysmorphic micromegakaryocytes are more prominent in blood and marrow.

TRANSITIONS BETWEEN IDIOPATHIC MYELOFIBROSIS AND OTHER MYELOPROLIFERATIVE DISORDERS

- Polycythemia vera. Approximately 15% of patients treated with phlebotomy or myelosuppression develop classic myelofibrosis (akin to primary myelofibrosis), usually in a progressive pattern over a period of many years (see Chap. 41).
- Apparent primary thrombocytosis may evolve into classic primary myelofibrosis (see Chap. 42).
- Acute leukemia develops in about 5% to 10% of patients with de novo primary myelofibrosis, and in about 20% of those who have transformed into primary myelofibrosis from a preceding case of polycythemia vera, especially those who have been treated with myelosuppressive agents. An oligoblastic myelogenous leukemia may precede the development of florid acute leukemia.

TREATMENT

- Some patients are asymptomatic for long periods of time and do not require therapy. There is currently no cure other than allogeneic hematopoietic stem cell transplantation, which may be difficult if fibrosis is extensive and at advanced age.
- Severe anemia may improve with androgen therapy in some patients (eg, danazol, 600 mg/d, orally, may be less virilizing). Careful monitoring of hepatic function and periodic liver imaging with ultrasound to detect liver tumors are essential.
- Glucocorticoids (prednisone 25 mg/m^2 per day, orally, for a limited period) may occasionally be helpful in patients with significant hemolytic anemia. High-dose glucocorticoids have been reported to ameliorate primary myelofibrosis in children, but their secondary effects are severe and they should not be used for prolonged periods, if possible.
- Few patients have such inappropriately low serum erythropoietin as to indicate a recombinant human erythropoietin product would be useful for treatment of anemia.
- Decreases in splenic and hepatic size, improvement in constitutional symptoms (fever, bone pain, weight loss, night sweats), improvement in blood counts (increase in hemoglobin, decrease in elevated platelet counts), and decreased marrow fibrosis can occasionally be obtained with low doses of oral hydroxyurea (eg, 0.5–1.0 g/d or 1.0–2.0 g three times per week). Blood counts at appropriate intervals to allow dosage adjustment is important (eg, every week, then every 2 weeks, then every month).
- Patients with myelofibrosis tend to have a lower tolerance to chemotherapy than patients with other clonal myeloid diseases, and dosage should be lowered accordingly until tolerance is determined.
- Interferon-α has been useful in treating splenic enlargement, bone pain, and thrombocytosis.
- Inhibitors of JAK2 (eg, ruxolitinib) can provide dramatic benefits. Most often they lead to a decrease in spleen size and a lessening of constitutional symptoms. Thrombocytopenia may be

dose-limiting (see Table 47–2). They may benefit patients without a *JAK2* mutation, perhaps because they inhibit JAK isoforms that contribute to cytokine release.

- Lenalidomide can induce marked improvement in hemoglobin concentration, increase in platelet count, and decrease in spleen size in about 25% to 30% of patients treated.
- Bisphosphonates (eg, etidronate, 6 mg/d on alternate months) may relieve bone pain from osteosclerosis and periostitis and may improve hematopoiesis.
- Selective low-dose radiation therapy may be useful for:
  — Severe splenic pain or massive splenic enlargement (with contraindication to splenectomy such as thrombocytosis)
  — Ascites from peritoneal extramedullary hematopoiesis
  — Focal areas of severe bone pain from periostitis or osteolysis of a fibrohematopoietic tumor)
  — Extramedullary tumors, especially of the epidural space
- Allogeneic hematopoietic stem cell transplantation can be efficacious in patients with a suitable donor and features felt to be compatible with a good outcome from transplantation (eg, acceptable age range, absence of comorbidities, and other features).
- Nonmyeloablative allogeneic hematopoietic stem cell transplantation may increase age at which transplantation may be useful.
- Major indications for splenectomy:
  — Very painful, enlarged spleen unresponsive to drug or local radiation treatment
  — Excessive red cell and/or platelet transfusion requirement
  — Refractory hemolysis
  — Severe, symptomatic thrombocytopenia
  — Portal hypertension
- Patients with prolonged bleeding times, prothrombin times, or partial thromboplastin times are at serious risk for bleeding during and after surgery. They require meticulous preoperative evaluation and replacement therapy, surgical hemostasis, and postoperative care.
- Postsplenectomy morbidity is about 30% and mortality about 10%.
- Splenectomy for primary myelofibrosis has special difficulties because of large size, the adherence to neighboring structures (eg, inferior surface of diaphragm), prominent collateral circulation, and dilated splenoportal arteries.
- The morbidity and mortality from splenectomy and the minimal evidence for prolongation of life has led to conservatism in using this approach. It can, however, be helpful in carefully selected patients.
- Portal hypertension as a result of increased splenic blood flow may be improved by splenectomy, especially if the hepatic wedge pressure is elevated as a result of a large splenic blood flow to the liver. In patients with portal hypertension resulting from an intrahepatic block or hepatic vein thrombosis and a hepatic venous pressure gradient well above the normal of less than 6 torr, a splenorenal shunt can be performed. To avoid abdominal surgery, a transjugular intrahepatic portosystemic shunt can be used.
- Anagrelide may be useful for postsplenectomy thrombocytosis.

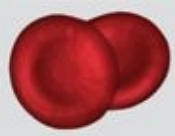
TABLE 47–2 **GUIDELINES FOR INITIAL ORAL RUXOLITINIB DOSE IN PRIMARY MYELOFIBROSIS**

Platelet Count	Dose
200×10^9/L	20 mg twice daily
$100–200 \times 10^9$/L	15 mg twice daily
$50–100 \times 10^9$/L	5 mg twice daily (increasing each month by 5 mg daily until maximal splenic size reduction, only if platelet count stays above 40×10^9/L)*

*Drug not FDA approved for starting platelet counts of 50 to 100×10^9/L.
If platelet count decreases while on ruxolitinib therapy, dose reduction should be made in relation to level of platelet count. The drug should not be administered if platelet counts falls to less than 50×10^9/L. Therapists should consult more detailed guidelines, *Prescribing Information*, published by Incyte, for use of ruxolitinib (Jakafi) (revised November 2011).
Source: *Williams Hematology*, 9th ed, Chap. 86, Table 86–4.

COURSE AND PROGNOSIS

- Median survival is approximately 5 years after diagnosis (range: 1 to 15 years).
- At least 16 variables have been associated with a less favorable prognosis.
- Six relatively consistent poor prognostic signs are (1) older age; (2) severity of anemia; (3) exaggerated leukocytosis ($> 25 \times 10^9$/L) or leukopenia ($< 4.0 \times 10^9$/L); (4) fever, sweating, or weight loss at the time of diagnosis; (5) a higher proportion of blast cells in blood (> 1.0%); and (6) cytogenetic abnormalities involving chromosomes 5, 7, or 17.
- Major causes of death are infection, hemorrhage, postsplenectomy mortality, and transformation into acute leukemia.
- Spontaneous remissions are rare.



For a more detailed discussion, see Marshall A. Lichtman and Josef T. Prchal: Primary Myelofibrosis, Chap. 86 in *Williams Hematology*, 9th ed.

PART VII

THE POLYCLONAL LYMPHOID DISEASES

CHAPTER 48

Classification and Clinical Manifestations of Polyclonal Lymphocyte and Plasma Cell Disorders

CLASSIFICATION

- Polyclonal lymphocyte and plasma cell disorders can be classified into two major groups: primary disorders and acquired disorders. See **Table 48–1**.
  - — Primary disorders result from defects intrinsic to B lymphocytes (eg, X-linked agammaglobulinemia), T lymphocytes (eg, congenital thymic aplasia), and/or natural killer cells, the latter usually coupled with a B- or T-cell deficiency (eg, interleukin-7 receptor α-chain deficiency) (see Chap. 50).
  - — Acquired disorders result from physiologic or pathophysiologic responses to extrinsic factors, usually infectious agents (eg, Epstein-Barr virus or human immunodeficiency virus infection) (see Chaps. 51 and 52).
- Monoclonal (neoplastic) lymphocyte and plasma cell disorders are discussed in Part VIII and are classified in Chap. 53. Specific neoplastic disorders are discussed in Chaps. 54 to 71.
- Lymphocyte disorders can have clinical manifestations that are not restricted to cells of the immune system (eg, leprosy or systemic lupus erythematosus).
- In some cases, classification is influenced by disease manifestations:
  - — Diseases caused by production of pathologic autoantibodies (eg, autoimmune hemolytic disease [see Chap. 22–24] and autoimmune thrombocytopenia [see Chap. 73]).
  - — Diseases caused by excess production of lymphocyte cytokines (eg, chronic inflammatory disorders).

TABLE 48–1 CLASSIFICATION OF NONCLONAL DISORDERS OF LYMPHOCYTES AND PLASMA CELLS

I. **Primary disorders**
  A. B-lymphocyte deficiency or dysfunction
    1. Agammaglobulinemia
      a. Acquired agammaglobulinemia
      b. Associated with plasma cell myeloma, heavy chain disease, light chain amyloid, Waldenström macroglobulinemia, or chronic lymphocytic leukemia
      c. Associated with celiac disease
      d. X-linked agammaglobulinemia of Bruton
      e. Autosomal recessive agammaglobulinemia
      f. Common variable immunodeficiency
      g. Transient hypogammaglobulinemia of infancy
      h. Bloom syndrome
      i. Comel-Netherton syndrome
    2. Selective agammaglobulinemia
      a. Immunoglobulin (Ig) M deficiency
        (1) Selective IgM deficiency

(2) Wiskott-Aldrich syndrome
b. Selective IgG deficiency
c. IgA deficiency
(1) Isolated asymptomatic
(2) Steatorrheic
d. IgA and IgM deficiency
e. IgA and IgG deficiency
(1) CD40/CD40L deficiency
(2) Activation-induced cytidine deaminase (AID) (uracil-DNA glycosylate [UNG], hyper-IgM)
(3) *PMS2* deficiency
3. Hyper-IgA
4. Hyper-IgD
5. Hyper-IgE
6. Hyper-IgE associated with HIV infection
7. Hyper-IgM immunodeficiency
8. X-linked lymphoproliferative disease

B. T-lymphocyte deficiency or dysfunction
1. Cartilage-hair hypoplasia
2. Lymphocyte function antigen-1 deficiency
3. Thymic aplasia (DiGeorge syndrome)
4. Thymic dysplasia (Nezelof syndrome)
5. Thymic hypoplasia
6. CD8 deficiency
7. CD3γ deficiency
8. Winged helix deficiency (Nude)
9. Interleukin-2 receptor α chain (CD25) deficiency
10. Signal transducer and activator of transcription 5b (STAT 5b) deficiency
11. Schimke syndrome
12. Janus kinase 3(JAK3) deficiency
13. γc Deficiency
14. Wiskott-Aldrich syndrome
15. Zeta-associated protein of 70kDa (ZAP-70) deficiency
16. Purine nucleoside phosphorylase deficiency
17. Interleukin-7 receptor deficiency
18. Major histocompatibility complex class I or II deficiency
19. Coronin-1A deficiency
20. IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome caused by mutations in FoxP3 that cause a deficiency of CD4+ regulatory T cells (T_{regs})
21. APECED (autoimmune polyglandular, candidiasis, and ectodermal dystrophy) syndrome caused by mutations in the autoimmune regulator gene (AIRE) gene
22. Autoimmune lymphoproliferative syndrome

C. Combined T- and B-cell deficiency or dysfunction
1. Ataxia-telangiectasia
2. Combined immunodeficiency syndrome
a. Adenosine deaminase deficiency
b. Thymic alymphoplasia
c. CD45 deficiency
d. X-linked severe combined immunodeficiency syndrome
3. Major histocompatibility complex class II deficiency—bare lymphocyte syndrome
4. IgG and IgA deficiency and impaired cellular immunity (type I dysgammaglobulinemia)
5. Thymoma-association immunodeficiency
6. Pyridoxine deficiency
7. Reticular agenesis (congenital aleukocytosis)
8. Omenn syndrome
9. Warts, hypogammaglobulinemia, infections, myelokathexis (WHIM) syndrome resulting from mutation in the CXCR4 gene

D. Natural killer cells: chronic natural killer cell lymphocytosis

II. **Acquired disorders**

A. Acquired immunodeficiency syndrome (AIDS)

B. Reactive lymphocytosis or plasmacytosis
1. *Bordetella pertussis* lymphocytosis
2. Cytomegalovirus mononucleosis
3. Drug-induced lymphocytosis
4. Stress-induced lymphocytosis
5. Persistent polyclonal B-cell lymphocytosis
6. Postsplenectomy lymphocytosis
7. Epstein-Barr virus mononucleosis
8. Inflammatory (secondary) plasmacytosis of marrow
9. Large granular lymphocytosis
10. Other viral mononucleosis
11. Polyclonal lymphocytosis
12. Serum sickness
13. T-cell lymphocytosis associated with thymoma
14. *Toxoplasma gondii* mononucleosis
15. Trypanosoma cruzi
16. Viral infectious lymphocytosis
17. Cat-scratch and other chronic bacterial infection

C. T-lymphocyte dysfunction or depletion associated with systemic disease
1. B-cell chronic lymphocytic leukemia
2. Hodgkin lymphoma
3. Leprosy
4. Lupus erythematosus
5. Sjögren syndrome
6. Sarcoidosis

Source: *Williams Hematology,* 9th ed, Chap. 78, Table 78–1.

CLINICAL MANIFESTATIONS

B-Lymphocyte Disorders

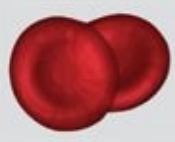
- Infection with any class of microorganism (eg, bacteria, viruses, fungi) occurs because of immunoglobulin deficiency and impaired opsonization and clearance of pathogen.
- Tissue or organ abnormality is a result of pathogenic autoantibodies (eg, immune hemolytic anemia, immune thrombocytopenia, myasthenia gravis, thyroiditis).
- Primary defect in the B-cell clone or expansion of a clone in response to chronic antigen stimulations can result in excess production of immunoglobulin that in turn produces a monoclonal gammopathy (Chap. 67).

T-Lymphocyte Disorders

- T-cell depletion results in immune deficiency.
- Clinical manifestations depend on the subset(s) of T cells involved:
  — *Depletion of T_H1-type $CD4^+$ T cells.* Impaired delayed-type hypersensitivity can lead to an increased risk of opportunistic infections (eg, mycobacteria, listeria, brucella, fungi, or other intracellular organisms) as a result of the deficient cellular immune response to these organisms.
  — *Depletion of T_H2-type $CD4^+$ T cells.* This is an impaired secondary antibody response to bacteria, viruses, and fungi.
  — *Depletion of $CD4^+$ regulatory T-cells.* Systemic autoimmune diseases can occur as a result.

— *Depletion of $CD4^+$ Th17 in skin and gastrointestinal tract*. This can lead to increased risk of infection in those sites.

- Graft-versus-host disease, mediated by T lymphocytes, is usually secondary to allogeneic hematopoietic cell transplantation (see Chap. 39).



For a more detailed discussion, see Yvonne A. Efebera and Michael A. Caligiuri: Classification and Clinical Manifestations of Lymphocyte and Plasma Cell Disorders, Chap. 78 in *Williams Hematology*, 9th ed.

CHAPTER 49
Lymphocytosis and Lymphocytopenia

LYMPHOCYTOSIS

Definition

- In adults, the absolute lymphocyte count exceeds 4.0×10^9/L.
- Normal lymphocyte count in childhood is higher than adults (mean ~6.0×10^9/L) (see Chap. 1).
- **Table 49–1** lists conditions associated with lymphocytosis.
- Examine blood film to determine if there is abnormal prevalence of:
  - — Reactive lymphocytes, associated with infectious mononucleosis (see Chap. 52)
  - — Large granular lymphocytes, associated with large granular lymphocyte leukemia (see Chap. 57)
  - — Small lymphocytes and smudge cells, associated with chronic lymphocytic leukemia (CLL) (see Chap. 55)
  - — Small cleaved lymphocytes, associated with low- or intermediate-grade lymphomas (see Chap. 61)
  - — Blasts, associated with acute lymphocytic leukemia (see Chap. 54)
- Several key tests permit discrimination between polyclonal and monoclonal disorders. Flow cytometric immunophenotyping of cell surface markers (CD), serum protein electrophoresis and immunofixation for monoclonal immunoglobulins, studies of T-cell–receptor gene rearrangement, or clonal cytogenetic findings can distinguish monoclonal lymphocytosis (B or T lymphocytic leukemia or lymphoma) from polyclonal (reactive) lymphocytosis.

TABLE 49–1 CAUSES OF LYMPHOCYTOSIS

I. Primary lymphocytosis
  A. Lymphocytic malignancies
    1. Acute lymphocytic leukemia (Chap. 54)
    2. Chronic lymphocytic leukemia and related disorders (Chap. 55)
    3. Prolymphocytic leukemia (Chap. 54)
    4. Hairy cell leukemia (Chap. 56)
    5. Adult T-cell leukemia (Chaps. 54 and 66)
    6. Leukemic phase of B-cell lymphomas (Chaps. 60, 61)
    7. Large granular lymphocytic leukemia (Chaps. 57, 66)
      a. Natural killer (NK) cell leukemia (Chap. 66)
      b. CD8+ T-cell large granular lymphocytic leukemia (Chap. 66)
      c. CD4+ T-cell large granular lymphocytic leukemia (Chap. 66)
      d. γ/δ T-cell large granular lymphocytic leukemia (Chap. 66)
  B. Monoclonal B-cell lymphocytosis (Chap. 55)
  C. Persistent polyclonal B cell lymphocytosis

II. Reactive lymphocytosis
  A. Mononucleosis syndromes (Chap. 52)

1. Epstein-Barr virus
2. Cytomegalovirus
3. Human immunodeficiency virus
4. Herpes simplex virus type II
5. Rubella virus
6. Toxoplasma gondii
7. Adenovirus
8. Infectious hepatitis virus
9. Dengue fever virus
10. Human herpes virus type 6 (HHV-6)
11. Human herpes virus type 8 (HHV-8)
12. Varicella zoster virus

B. *Bordetella pertussis*
C. NK cell lymphocytosis (see Chap. 57)
D. Stress lymphocytosis (acute)
1. Cardiovascular collapse
a. Acute cardiac failure
b. Myocardial infarction
2. Staphylococcal toxic shock syndrome
3. Drug-induced
4. Major surgery
5. Sickle cell crisis
6. Status epilepticus
7. Trauma

E. Hypersensitivity reactions
1. Insect bite
2. Drugs

F. Persistent lymphocytosis (subacute or chronic)
1. Cancer
2. Cigarette smoking
3. Hyposplenism
4. Chronic infection
a. Leishmaniasis
b. Leprosy
c. Strongyloidiasis
5. Thymoma

Source: *Williams Hematology*, 9th ed, Table 79–1.

Primary Clonal Lymphocytosis

- Neoplastic (monoclonal) proliferation of B cells, T cells, or natural killer (NK) cells
- Monoclonal B-cell lymphocytosis (see Chap. 55)
  — There are no associated clinical manifestations.
  — Some patients may develop CLL or another type of progressive lymphoproliferative disease (see Chap. 55).
- Chronic natural killer (NK) cell lymphocytosis (see Chap. 57)
  — CD3–CD16+CD56+ lymphocytes are present.
  — Approximately 60% of cases have no other signs or symptoms.
  — Others may have blood cytopenias, especially neutropenia, neuropathy, and splenomegaly.
- Acute and chronic lymphocytic leukemias and lymphomas with blood involvement (see Chap. 53)

Primary Polyclonal Lymphocytosis

- Persistent polyclonal lymphocytosis of B lymphocytes
  — A high proportion of lymphocytes have bilobed nuclei or have other nuclear abnormalities (**Figure 49–1**).
  — Lymphocytes are "polyclonal" in their expression of immunoglobulin (**Figure 49–1**).
  — This is commonly associated only with mild splenomegaly and/or raised serum IgM but can have features that resemble those of patients with monoclonal B-cell malignancies.
  — Most patients have small numbers of B cells with chromosomal abnormalities. These most commonly involve chromosomes 3, 14 (at the immunoglobulin heavy chain locus), and 18 (at the *BCL-2* locus).
  — This condition is generally not progressive, although some cases may evolve into a monoclonal lymphoproliferative disease.

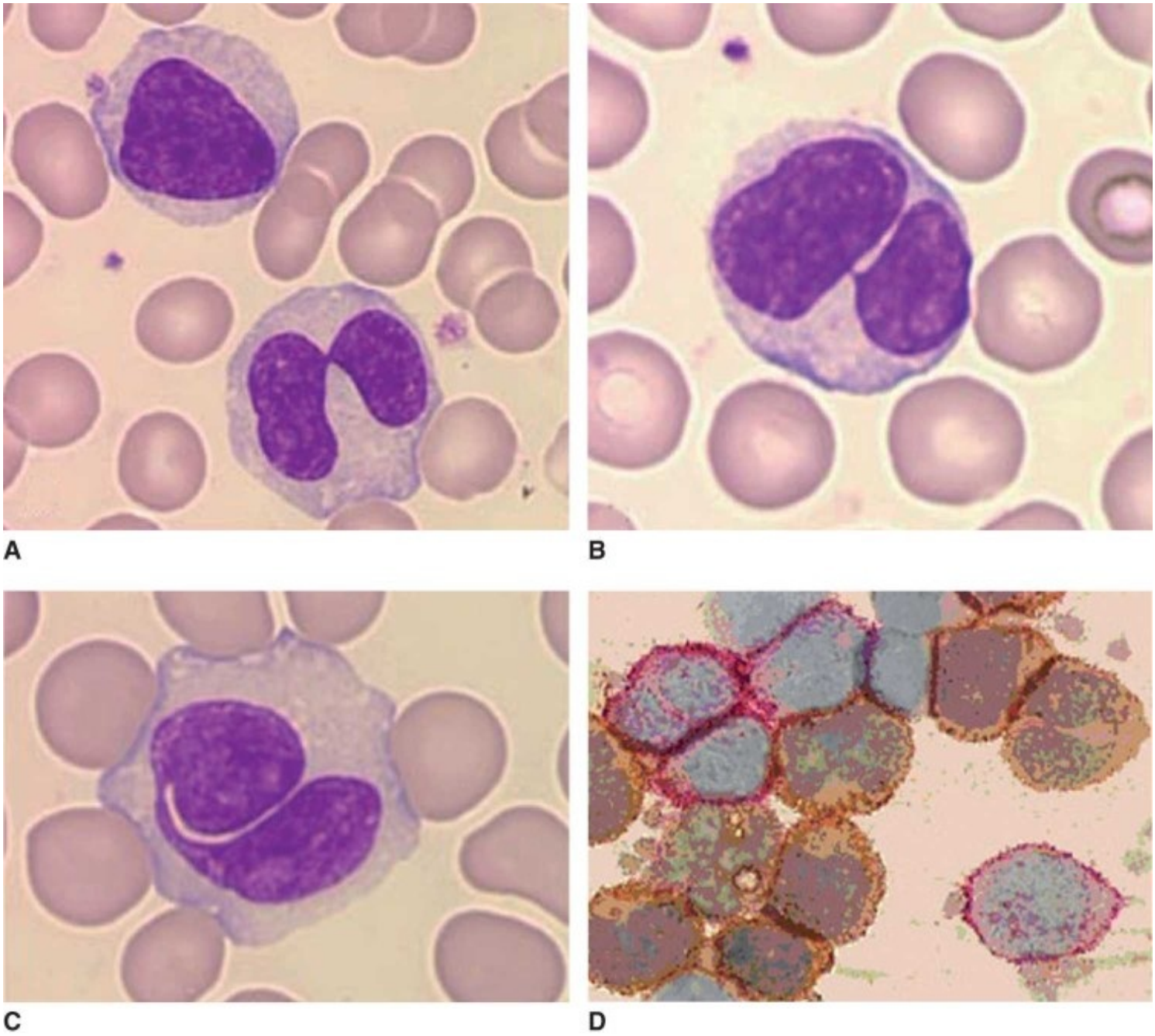


FIGURE 49–1 Persistent polyclonal lymphocytosis of B lymphocytes. Blood film. **A–C.** Examples of the nuclear abnormality of lymphocytes in this disorder. The lymphocyte nucleus may be bilobed or segmented although not fully bilobed. Some are monolobed. **D.** Light-chain analysis. Immunoenzymatic method. Cytocentrifuge cell preparation. Anti-kappa immunoglobulin light chain tagged

with peroxidase and anti-lambda light chain tagged with alkaline phosphatase. Note polyclonal reactivity of lymphocytes; some cells with surface kappa light chains (*brownish*) and some with surface lambda light chains (*reddish*). Molecular studies did not show immunoglobulin gene rearrangement. (Used with permission from Dr. Xavier Troussard, Laboratoire d'Hématologie CHU Côte de Nacre, Caen, France.

Secondary (Reactive) Lymphocytosis

- Lymphocytosis secondary to a physiologic or pathophysiologic response to infection of B lymphocytes, toxins, cytokines, or unknown factors

Infectious Mononucleosis

- This disorder is caused by the Epstein-Barr virus infection (see Chap. 52).
- Lymphocytosis is principally from a polyclonal increase in CD8+ T lymphocytes.
- Characteristic reactive lymphocytes are evident on blood film (see **Figure 49–2**).

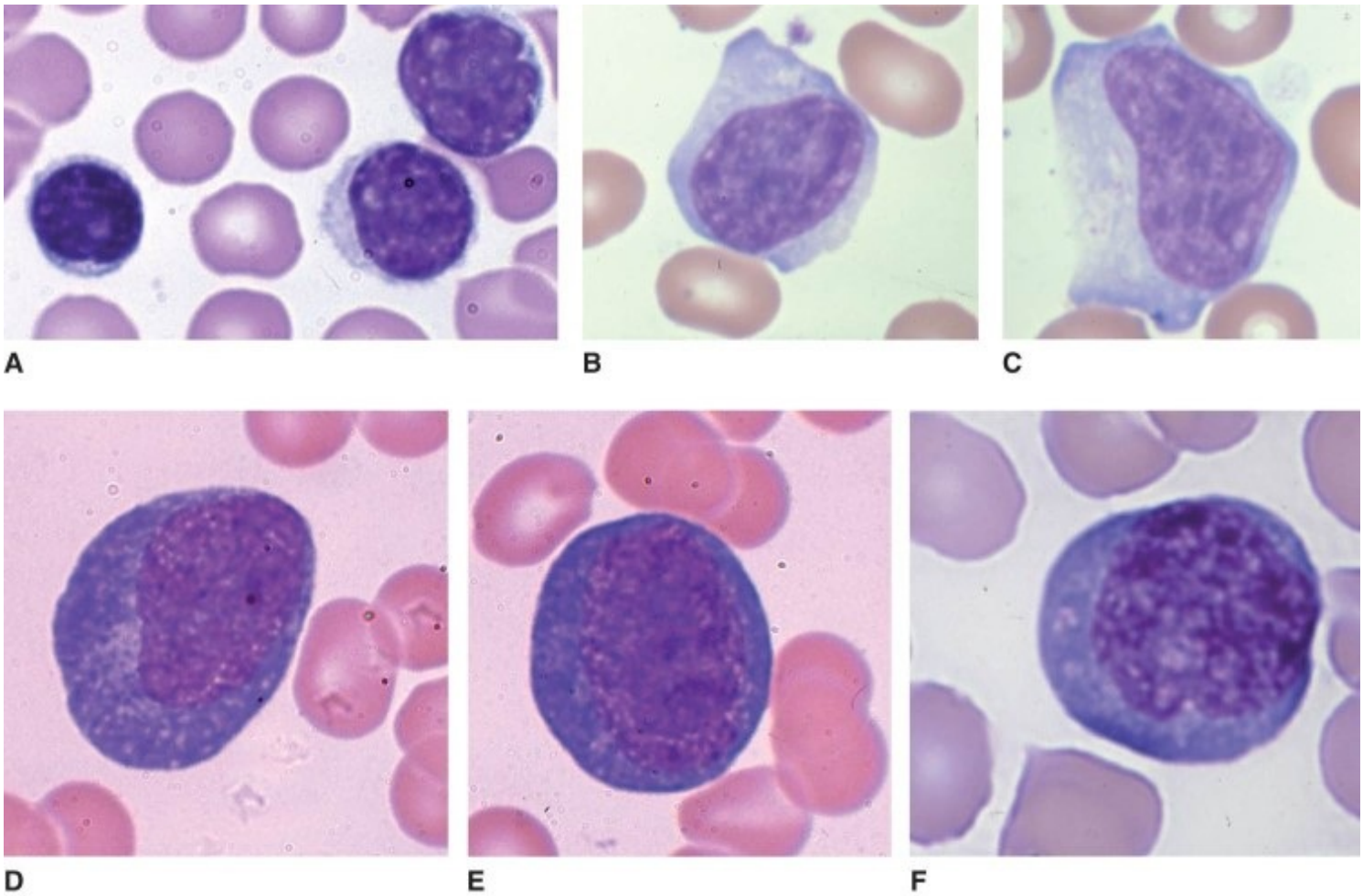


FIGURE 49–2 Blood films. **A.** Acute infectious lymphocytosis. The lymphocytosis in this disorder of childhood is composed of normal-appearing lymphocytes, which may vary somewhat in size as shown in the blood of this case. Note typical small lymphocyte with dense chromatin pattern and scant rim of cytoplasm and somewhat two larger lymphocytes with less dense chromatin pattern. **B, C.** Reactive lymphocytes. Large lymphocytes with an increased proportion of cytoplasm with basophilic cytoplasmic edges, often engaging neighboring red cells. Nucleoli may occasionally be evident. This variation in lymphocyte appearance can occur in a variety of disorders that provoke an immunologic response, including viral illnesses. They are indistinguishable in appearance by light microscopy from the reactive lymphocytes seen in infectious mononucleosis, viral hepatitis, or other conditions such as Dengue fever. **D–F.** Plasmacytoid lymphocytes. In this type of reactive lymphocytosis, the lymphocytes are large and have deep blue-colored cytoplasm, approaching the coloration of plasma cell cytoplasm, but they retain the nuclear appearance, cell shape, and cell size of a medium-size lymphocyte, and they do not develop a prominent paranuclear clear zone or markedly eccentric nuclear position as do most plasma cells. They may be seen in a variety of situations, including infections, drug hypersensitivity, and serum

sickness-type reactions. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

Lymphocytosis Induced by Other Infectious Diseases

- This disease is usually viral (eg, hepatitis A virus).
- It is characterized morphologically by reactive lymphocytes (see **Figure 49–2**).

Acute Infectious Lymphocytosis

- This childhood disease usually affects individuals aged 2 to 10 years.
- It is characterized by marked lymphocytosis of polyclonal morphologically normal T lymphocytes and NK cells (see **Figure 49–2**).
- Although an infection of unknown etiology, some cases have been associated with acute infection by coxsackievirus B2, toxoplasmosis, or falciparum malaria.
- The disease is usually asymptomatic, but fever, abdominal pain, or diarrhea may be present for a few days.
- No enlargement of liver or spleen occurs.
- Lymphocytosis is usually 20 to 30 $\times 10^9$/L but can be as high as 100 $\times 10^9$/L.
- Lymphocytosis may persist for several weeks after clinical symptoms have subsided.
- Marrow shows variable increase in number of lymphocytes.
- Serum is negative for heterophil antibodies.

Bordetella pertussis Infection

- Lymphocytosis of morphologically normal CD4+ T cells occurs, ranging from 8 to 70 $\times 10^9$/L.
- Lymphocytosis is caused by failure of lymphocytes to leave the blood because of pertussis toxin, an adenosine diphosphate (ADP)-ribosylase that inhibits chemokine receptor signaling.
- Characteristic clefted nuclei in a proportion of lymphocytes are usually evident.
- Pertussis toxin also may induce T-cell activation by binding to neuraminic acid residues of T-cell–surface glycoproteins.

Large Granular Lymphocytosis

- This type of lymphocytosis can result from increase in NK cells, CD8+ cells or, rarely, CD4+ cells.
- Most common form is result of expansion of CD3-CD16+CD56+ NK cells.
- Termed NK lymphocytosis, NK cell counts range from 4 to 15 $\times 10^9$/L.
- Expansion of NK cells or T cells may represent an exaggerated response to systematic infections and/or immune deregulation.
- It may be associated with rheumatoid arthritis (< 1.0% of cases).
- See Chap. 57.

Drug-Induced Lymphocytosis

- Dasatinib and ibrutinib are associated with lymphocytosis when used for chronic myelogenous leukemia and CLL, respectively.

Stress Lymphocytosis (Acute)

- Lymphocytosis appears promptly as a consequence of a redistribution of lymphocytes induced by adrenaline.
- Lymphocytosis, often greater than 5×10^9/L, reverts to normal or low levels within hours.
- This condition may be associated with trauma, surgery, acute cardiac failure, septic shock, myocardial infarction, sickle cell crisis, or status epilepticus.

Hypersensitivity Reactions

- Reactions to insect bites may be associated with a large granular lymphocytic lymphocytosis and lymphadenopathy.

Persistent Chronic Polyclonal Lymphocytosis

- Cancer:
  — Solid tumors
  — Thymoma, probably a result of release of thymic hormones by neoplastic thymic epithelium
- Postsplenectomy lymphocytosis.
  — Can persist for prolonged periods after splenectomy (eg, > 50 months)
- Chronic inflammatory diseases:
  — Systemic diseases associated with inflammation (eg, sarcoidosis, Wegener granulomatosis)
- Autoimmune diseases (eg, rheumatoid arthritis):
  — In rheumatoid arthritis patients with Felty syndrome (associated neutropenia), important to rule out T-cell large granular lymphocytic leukemia (see Chap. 57)
- Cigarette smoking:
  — Perhaps a polyclonal increase in CD4+ T cells and in B cells (some binuclear), especially in HLA-DR7-positive women
  — Possible resolution on cessation of smoking

LYMPHOCYTOPENIA

Definition

- Absolute lymphocyte count is less than 1.0×10^9/L.
- Usual cause is a decrease in CD4+ (helper) T cells because this cell type accounts for about half of all blood lymphocytes.
- **Table 49–2** lists the conditions associated with lymphocytopenia.

TABLE 49–2 CAUSES OF LYMPHOCYTOPENIA

I. Inherited causes
  A. Congenital immunodeficiency diseases (Chap. 50)
    1. Severe combined immunodeficiency disease
      a. Aplasia of lymphopoietic progenitor cells
      b. Adenosine deaminase deficiency
      c. Absence of histocompatibility antigens
      d. Absence of CD4+ helper cells
      e. Thymic alymphoplasia with aleukocytosis (reticular dysgenesis)

f. Mutations in genes required for T-cell development
2. Common variable immune deficiency
3. Ataxia-telangiectasia
4. Wiskott-Aldrich syndrome
5. Immunodeficiency with short-limbed dwarfism (cartilage-hair hypoplasia)
6. Immunodeficiency with thymoma
7. Purine nucleoside phosphorylase deficiency
8. Immunodeficiency with veno-occlusive disease of the liver

B. Lymphopenia resulting from genetic polymorphism

II. Acquired causes

A. Aplastic anemia (Chap. 3)

B. Infectious diseases

1. Viral diseases
a. Acquired immunodeficiency syndrome (Chap. 51)
b. Severe acute respiratory syndrome
c. West Nile encephalitis
d. Hepatitis
e. Influenza
f. Herpes simplex virus
g. Herpes virus type 6 (HHV-6)
h. Herpes virus type 8 (HHV-8)
i. Measles virus
j. Other
2. Bacterial diseases
a. Tuberculosis
b. Typhoid fever
c. Pneumonia
d. Rickettsiosis
e. Ehrlichiosis
f. Sepsis
3. Parasitic diseases: acute phase of malaria infection

C. Iatrogenic

1. Immunosuppressive agents
a. Antilymphocyte globulin therapy
b. Alemtuzumab (CAMPATH 1-H)
c. Glucocorticoids
2. High-dose psoralen plus ultraviolet A treatment
3. Stevens-Johnson syndrome
4. Chemotherapy
5. Platelet or stem cell apheresis procedures
6. Radiation
7. Major surgery
8. Extracorporeal bypass circulation
9. Renal or marrow transplant
10. Thoracic duct drainage
11. Hemodialysis
12. Apheresis for donor lymphocyte infusion

D. Systemic disease associated

1. Autoimmune diseases
a. Arthritis
b. Systemic lupus erythematosus
c. Sjögren syndrome
d. Myasthenia gravis
e. Systemic vasculitis
f. Behçet-like syndrome
g. Dermatomyositis
h. Wegener granulomatosis
2. Hodgkin lymphoma (Chap. 59)
3. Carcinoma

4. Idiopathic myelofibrosis
5. Protein-losing enteropathy
6. Heart failure
7. Sarcoidosis
8. Thermal injury
9. Severe acute pancreatitis
10. Strenuous exercise
11. Silicosis
12. Celiac disease

E. Nutritional and dietary
1. Ethanol abuse
2. Zinc deficiency

III. Idiopathic: idiopathic CD4+ T lymphocytopenia

Source: *Williams Hematology,* 9th ed, Table 79–3.

Inherited Causes

- This stem cell abnormality, either quantitative or qualitative, results in ineffective lymphopoiesis (see Chap. 50).
- Other abnormalities, such as the Wiskott-Aldrich syndrome, result in premature destruction of T cells because of cytoskeletal abnormalities (see Chap. 50).

Acquired Lymphocytopenia

Infectious Diseases

- Acquired immunodeficiency syndrome (AIDS); destruction of CD4+ (helper) T cells infected with human immunodeficiency virus-1 or -2 (HIV-1 or HIV-2) (see Chap. 51)
- Other viral diseases such as influenza
- Active tuberculosis; resolution of lymphocytopenia usually 2 weeks after initiating appropriate therapy

Iatrogenic

- Radiotherapy, chemotherapy, or administration of antilymphocyte globulin or alemtuzumab (CAMPATH-1H)
- Treatment of psoriasis with psoralen and ultraviolet A irradiation, which may result in T-lymphocyte lymphopenia
- Glucocorticoid therapy; mechanism unclear, possibly involving redistribution as well as cell destruction
- Major surgery, possibly from redistribution of lymphocytes
- Thoracic duct drainage, because lymphocytes are removed from the body
- Platelet apheresis, because lymphocytes, as well as platelets, are removed from the body, resulting in transient lymphopenia

Systemic Disease Associated with Lymphocytopenia

- Systemic lupus erythematosus that is probably mediated by autoantibodies
- Sarcoidosis, probably a consequence of impaired T-cell proliferation
- Protein-losing enteropathy, in which lymphocytes may be lost from the body

Burns

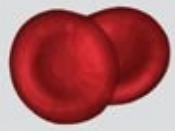
- Profound T-cell lymphocytopenia caused by redistribution from blood to tissues

Nutritional/Dietary Factors

- Zinc deficiency (Zinc is necessary for normal T-cell development and function.)
- Excess alcohol intake, which may impair lymphocytic proliferation

Idiopathic CD4+ T Lymphocytopenia

- This condition is defined as a CD4+ T-lymphocyte count less than 3×10^8/L on two separate occasions without serologic or virologic evidence of HIV-1 or HIV-2 infection.
- Congenital immunodeficiency diseases, such as common variable immunodeficiency, should be excluded (see Chap. 50).
- Decrease of CD4+ cell counts is generally gradual.
- More than half of reported cases had opportunistic infections indicative of cellular immune deficiency (eg, *Pneumocystis jiroveci* pneumonia). Such patients are classified as having idiopathic CD4+ T-lymphocytopenia and severe unexplained HIV-seronegative immune suppression. In contrast to patients infected with HIV, these patients generally have stable CD4+ counts over time and reductions in other lymphocyte subgroups, and they may experience complete or partial spontaneous reversal of the CD4+ T lymphocytopenia.



For a more detailed discussion, see Sumithira Vasu and Michael A. Caligiuri: Lymphocytosis and Lymphocytopenia, Chap. 79 in *Williams Hematology*, 9th ed.

CHAPTER 50
Primary Immunodeficiency Syndrome

- Primary immune deficiency diseases (PIDDs) are characterized by increased susceptibility to infections and result from the failure of either the humoral or cellular arms of the immune system or both.
- The clinical features of PIDDs are listed in **Table 50–1**.
  — PIDDs are characterized by recurrent pyogenic bacterial infections, including sinusitis, furunculosis, and recurrent or chronic pneumonias that often terminate in bronchiectasis. These infections are initially responsive to antibiotics but soon recur.
- Evaluation of serum immunoglobulin (Ig) levels and specific antibody responses in patients with recurring infections without an apparent predisposing cause should be made.
  — Baseline Ig levels are often low or virtually absent.
  — Antibody response to immunization is often inadequate.
- Abnormality of cellular immunity causes:
  — Susceptibility to viral, protozoal, and fungal infections may occur.
  — Patients are often anergic. Rejection or clearance of allogeneic cells may be impaired.
  — There may be a secondary defect in humoral immunity because of T-cell dysfunction and loss of B-cell helper activity.
- Autoimmune diseases such as immune-mediated hemolytic anemia, thrombocytopenia, or rheumatoid arthritis-like conditions occur at a higher frequency in certain primary immunodeficiency states.
- The more severe primary immune deficiencies are usually present in infancy, although common variable immunodeficiency (CVID) often presents later in life.
- In Ig-deficient patients, treatment with intravenous immunoglobulin (IVIG) may decrease infectious events.
- The autosomal recessive syndromes are frequently the result of consanguineous marriages.

TABLE 50–1 CLINICAL FEATURES OF PRIMARY IMMUNODEFICIENCY DISORDERS

Neutrophils Numerical or Functional Defects	Complement Deficiencies	Antibody Deficiencies	Combined Immune Deficiencies
Severe bacterial and fungal infections Skin or deep bacterial and fungal abscesses Infections sustained by unusual bacteria and fungi	Recurrent or severe infections sustained by encapsulated pathogens Recurrent *Neisseria meningitidis* infections Autoimmune manifestations (SLE-like) Atypical hemolytic uremic syndrome Recurrent angioedema (C1-INH deficiency)	Recurrent infections after 4–6 months of age Intestinal *Giardia lamblia* infection Enterovirus meningoencephalitis	Early-onset respiratory and gut infections Opportunistic infections Growth failure Persistent candidiasis Erythroderma

C1-INH, C1 esterase inhibitor; SLE, systemic lupus erythematosus.
Source: *Williams Hematology*, 9th ed, Chap. 80, Table 80–1.

PREDOMINANT ANTIBODY DEFICIENCIES

X-Linked and Autosomal Recessive Agammaglobulinemia

Definition and Genetic Features

- This deficiency is caused by a maturation defect in B-cell development.
- X-linked agammaglobulinemia is the result of a mutation in the Bruton tyrosine kinase (*BTK*) gene.
- Autosomal recessive agammaglobulinemia is the result of mutations in genes relevant to immunoglobulin heavy or light chains (ie, *IGHM*, *IGLL1*, *CD79a*, *CD79b* or the B-cell adaptor molecule, *BLINK*).

Clinical Features

- X-linked and autosomal recessive agammaglobulinemia have similar clinical features: low Ig levels, decreased B cells, and recurrent infections.
- Normal levels of IgG at birth occur as a result of transfer from maternal circulation. Thus, affected individuals are usually asymptomatic for the first few months of life.
- Symptoms and signs vary and may be mild or severe (see **Table 50–1**). The condition first develops between 4 and 12 months of age but may not be apparent for several years in some patients.
- Otitis media, sinusitis, pyoderma, and diarrhea are the most frequent presenting findings.
- Pneumonia, meningitis, septicemia, osteomyelitis, and septic arthritis may occur later.
- Neutropenia may accompany X-linked agammaglobulinemia and increase the risk of recurrent or chronic infections.
- The most common pathogens are *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Staphylococcus aureus*.
- The response to many viral infections is often normal, but patients are susceptible to echovirus and coxsackie virus presenting as meningoencephalitis, dermatomyositis, or hepatitis. They are also unusually susceptible to poliovirus. Attenuated live polio vaccine can cause severe morbidity and mortality once maternal antibodies have disappeared.
- Gastroenteritis caused by *Giardia lamblia*, *Campylobacter* species, or rotavirus is common and may be associated with malabsorption.
- Patients have an increased incidence of aggressive rectosigmoid carcinoma.

Laboratory Features

- Reduced level of serum Ig is the hallmark; B cells are less than 1% of normal (tonsils are absent).
- B-cell maturation arrest prevents plasma cell development, and these cells are absent from the marrow, gastrointestinal tract, and lymph nodes.
- Specific antibodies are also reduced or absent (**Table 50–2**).

- Flow cytometry can be used to measure the BTK protein in normal monocytes or platelets and female carriers can be identified.
- Analysis of mutations in the *BTK* gene can be used to make the diagnosis and to identify affected males in utero.

TABLE 50–2 COMMON ADAPTIVE IMMUNODEFICIENCIES: LABORATORY AND CLINICAL FEATURES

	Lymphocytes*				Humoral Immunity: Serum Immunoglobulins (Ig)					
	B	T	NK	Cellular Immunity	M	G	A	E	Antibody Responses	Common Infections
Predominantly antibody deficiencies										
X-linked agammaglobulinemia BTK deficiency	–	+	+	+	↓	↓	↓	↓	–	Bacteria, *Giardia lamblia*
Autosomal recessive agammaglobulinemia λ5, Igα, Igβ, BLNK, p85α, E47 deficiency	–	+	+	+	↓	↓	↓	↓	–	Bacteria
Transient hypogammaglobulinemia of infancy	+	+	+	+	N/↓	N/↓	N/↓	N/↓	+/–	Bacteria
Selective IgA deficiency	+	+	+	+	N	N	↓	N	+/–	Bacteria, *G lamblia*
Common variable immune deficiency (CVID)	+	+	+	+	N/↓	↓	↓	↓	–	Bacteria, *G lamblia*
Hyper-IgM syndromes										
CD40 ligand deficiency (X-linked)	+	+	+	+/–	N/↑	↓	↓	↓	+/–	Bacteria, viruses, fungi
CD40 deficiency	+	+	+	+	N/↑	↓	↓	↓	+/–	Bacteria, viruses, fungi
Activation-induced cytidine deaminase deficiency (AID)	+	+	+	+	N/↑	↓	↓	↓	+/–	Bacteria
Uracil-DNA glycosylase deficiency (UNG)	+	+	+	+	N/↑	↓	↓	↓	+/–	Bacteria
IKK-γ (NEMO) deficiency, X-linked	+	+	+	+	N/↑	↓	↓	↓	+/–	Bacteria, viruses, fungi
Severe combined immunodeficiencies (SCIDs)										
Interleukin receptor γ-chain deficiency (X-linked SCID)	+	–	–	–	N	↓	↓	↓	–	Bacteria, viruses, fungi
Janus-associated kinase 3 (JAK3) deficiency	+	–	–	–	N	↓	↓	↓	–	Bacteria, viruses, fungi
Interleukin-7 receptor α-chain deficiency	+	–	–	–	N	↓	↓	↓	–	Bacteria, viruses, fungi
ZAP-70 tyrosine kinase deficiency	+	+/–	+	–	N	N/↓	N/↓	N/↓	+/–	Bacteria, viruses, fungi
Adenosine deaminase (ADA) deficiency	–	–	–	–	↓	↓	↓	↓	–	Bacteria, viruses, fungi
Purine nucleotide phosphorylase (PNP) deficiency	+	–	+	–	N	↓	↓	↓	+/–	Bacteria, viruses, fungi
Recombinase activating genes (RAG 1/2) deficiency	–	–	+	–	↓	↓	↓	↓	–	Bacteria, viruses, fungi
Artemis deficiency	–	–	+	–	↓	↓	↓	↓	–	Bacteria, viruses, fungi
Reticular dysgenesis (AK2 deficiency)	–	–	–	–	↓	↓	↓	↓	–	Bacteria, viruses, fungi
Primary T-cell deficiencies										
Congenital thymic aplasia (DiGeorge syndrome)	+	–	+	+/–	N	N	N	N	+/–	Bacteria, viruses, fungi
MHC class II deficiency	+	+/–	+	+	N	↓	↓	↓	+/–	Bacteria, viruses, fungi
TAP-1, TAP-2 deficiency (MHC class I deficiency)	+	+/–	+/–	+	N	N	N	N	+	Bacteria, viruses, fungi
Other well-defined immunodeficiency syndromes										Bacteria
Ataxia-telangiectasia	+	+	+	+/–	N/↑	N/↓	N/↓	↓	+/–	
Wiskott-Aldrich syndrome	+	+/–	+	+/–	↓	N	↑	↑	+/–	Bacteria
Hyper-IgE Syndromes										
STAT3 deficiency (AD)	+/–	+	+	+/–	N	N	N	↑↑	+/–	*Staphylococcus aureus*, *Candida albicans*
DOCK8 deficiency (AR)	+/–	+/–	+/–	+/–	↓	N	N	↑↑	+/–	*C albicans*, viruses, fungi
GATA 2 deficiency (AD)	–	+	–	+/–	N	N	N	N	+/–	Mycobacteria, viruses, fungi
IPEX, IPEX-like	+	(lack of T^{reg}s)	+	+	N	N	↑	↑	+	Autoimmunity, *S aureus*, *C albicans*, cytomegalovirus

*Natural killer lymphocytes (NK), T cells (T), B cells (B).
Normal levels (+), reduced or absent levels (–); normal (N), elevated (↑), or reduced (↓) serum immunoglobulins.
Source: *Williams Hematology*, 9th ed, Chap. 80, Table 80–2.

Treatment

- IVIG with pooled plasma-derived preparation that contains IgG and IgA is used.
- Individualized doses usually start at 400 to 600 mg/kg intravenously every 4 weeks.
- Prophylactic antibiotic therapy may be useful in certain circumstances, such as in patients with chronic lung disease.

Course

- Treatment with IVIG markedly decreases enteroviral infections, but some patients develop neurodegenerative disease or chronic ileitis (Crohn-like disease) despite treatment and without evidence of an infectious etiology.

Hyperimmunoglobulin M Syndromes

Definition and Genetics Abnormalities

- These syndromes are characterized by recurrent infections associated with low serum levels of IgG, IgA, and IgE but normal or increased levels of IgM.
- Mutations affect genes involved in B-cell activation, class switch recombination (*CSR)*, and somatic hypermutation (*SHM)*.
- Mutations occur in genes encoding enzymes intrinsic to B cells (ie, *AID*, *UNG*, and the *NEMO* gene, the latter crucial for nuclear factor-κB activation).

X-Linked Hyper-IgM as a Result of CD40 Ligand (CD40L) Deficiency

- This deficiency is caused by mutations in *CD40L* distributed throughout the gene, resulting in nonfunctional CD40L protein.
- Normally, CD40L on surface of CD4+ T lymphocytes interacts with CD40 expressed constituently on B lymphocytes.

Clinical Features

- Characterized by recurrent bacterial infections in infants, this often presents with interstitial pneumonia caused by *Pneumocystis jiroveci*.
- Fifty percent of affected males also develop neutropenia.
- There is a high risk of developing chronic *Cryptosporidium* infections complicated by ascending cholangiolitis and chronic liver disease.
- Progressive neurodegeneration, as in X-linked agammaglobulinemia, can occur.

Laboratory Features

- Blood B-cell subsets are normal, but B cells are naive.
- There is defective lymph node germinal center development and severe deficiency in follicular dendritic cells.
- Response to specific antigens is reduced (see **Table 50–2**).
- Mild cases left untreated can lead to red cell aplasia as a result of chronic parvovirus B19 infection.

Treatment

- During infancy, treat with trimethoprim-sulfamethoxazole to prevent *P jiroveci* pneumonia.
- IVIG doses are similar to X-linked agammaglobulinemia to prevent chronic infections, including parvovirus.
- Allogeneic hematopoietic stem cell transplantation should be considered if an appropriate donor can be identified.

- Treat severe and persistent neutropenia with granulocyte colony-stimulating factor (G-CSF).

Autosomal Recessive Hyper-IgM with CD40 Mutations

- Clinical features are similar to ones in CD40L mutations.

Autosomal Recessive Hyper-IgM Syndrome Caused by an Intrinsic Defect

- Mutations of the activation-induced cytidine deaminase gene (*AID*) occur.
- Because of mild phenotype, this condition is often discovered later in life.

Clinical Features

- Recurrent bacterial infections that affect upper and lower respiratory tract occur.
- Enlarged tonsils and lymph nodes from marked follicular hyperplasia are present.
- T- and B-cell subset are normal but all CD27+ memory B cells fail to switch isotypes and express IgM and IgD.
- Treatment with IVIG prophylaxis is often associated with excellent long-term prognosis.

X-linked Anhydrotic Ectodermal Dysplasia with Immunodeficiency Caused by Mutations in Nuclear Factor-κB Essential Modulator (NEMO)

- This immunodeficiency is characterized by partial or complete absence of sweat glands, sparse hair growth, and abnormal dentition.
- Most patients present with bacterial infections, especially *S pneumoniae, S aureus,* and atypical mycobacteria. Twenty percent of infections are caused by viral infections.
- Approximately 20% of patients develop inflammatory bowel disease.
- Treatment with IVIG is useful but does not prevent the occurrence of serious complications.

CVID and Selective IgA Deficiency

- CVID is heterogeneous and may present at any age but usually occurs during adulthood.
- This disorder is characterized by hypogammaglobulinemia, impaired antibody responses, and recurrent bacterial infections.
- CVID occurs in conjunction with selective IgA deficiency, the most common primary immunodeficiency syndrome.
- Selective IgA deficiency may be the initial presenting finding in later CVID.
- Familial concordance occurs in approximately 20% of cases, and CVID and IgA deficiency can occur in the same family.

Clinical Features of CVID

- Characteristic features include recurring sinopulmonary infections and bacterial pneumonia.
- If untreated, it may lead to bronchiectasis and chronic lung disease.
- Lymphadenopathy and splenomegaly are common.
- Caseating granulomas of lung, spleen, liver, skin, and other tissues are damaging to organ function.
- Gastrointestinal complaints are frequent. Lymphoid hyperplasia of the small bowel results in a

syndrome mimicking chronic inflammatory bowel disease.
- Bowel disease is associated with *Giardia lamblia* or *Campylobacter* infections.
- Autoimmune disorders are common and may resemble rheumatoid arthritis, dermatomyositis, or scleroderma.
- Patients may develop autoimmune hemolytic anemia, autoimmune thrombocytopenia purpura, autoimmune neutropenia, pernicious anemia, and chronic active hepatitis.
- Despite normal B-lymphocyte levels and lymph node cortical follicles, patients have agammaglobulinemia that may be profound.
- Uncommonly, patients may have an associated thymoma.

Treatment and Course of CVID

- IVIG infusions and prophylactic antibiotics are beneficial but often insufficient to prevent serious complications.
- There is a marked increase in the risk of lymphoma, gastrointestinal cancers, and a variety of other cancers.
- Allogeneic hematopoietic stem cell transplantation is not recommended unless used to treat a secondary lymphoma.

Clinical Features and Treatment of Selective IgA Deficiency

- This deficiency is characterized by < 10 mg/dL of IgA.
- The frequency of occurrence differs greatly among ethnic groups; it is highest in Scandinavia and lowest in Asian populations.
- Fundamental defect is the failure of IgA-bearing B lymphocytes to mature into IgA-secreting plasma cells.
- Most persons remain healthy.
- If disease signs occur, they include recurrent sinopulmonary infections and atopic symptoms including allergic conjunctivitis, rhinitis, and eczema. Food allergies are common, and asthma is more refractory to symptomatic treatment.
- Symptomatic individuals have concomitant deficiency in IgG_2 and IgG_3 and poor responses to polysaccharide antigens.
- Chronic giardiasis, malabsorption, celiac disease, primary biliary cirrhosis, and pernicious anemia can occur.
- There is a higher incidence of rheumatoid arthritis, myasthenia gravis, thyroiditis, and systemic lupus erythematosus.
- No specific treatment is available.
- In patients with chronic pulmonary disease, selective prophylactic antibiotic therapy may be useful and where a defect in response to polysaccharide antigens is suspected or measured, prophylactic IVIG can be beneficial.

SEVERE COMBINED IMMUNODEFICIENCIES

Definition and History

- This heterogeneous group of genetic disorders is characterized invariably by a severe

impairment of T-lymphocyte development and function and a variable defect in either B or natural killer (NK) cells or both (**Figure 50–1**).

- Severe combined immunodeficiency (SCID) can be classified into four groups based on the associated immune cell deficiencies:
  — T–B+NK– SCID (most common type)
  — T–B+NK+ SCID
  — T–B–NK+ SCID
  — T–B–NK– SCID
- The incidence rate is 1 in 50,000 births.
- The most common form is inherited as an X-linked trait.
- Reconstitution of a functioning immune system by transplantation of histocompatible allogeneic hematopoietic stem cells may be life-saving.

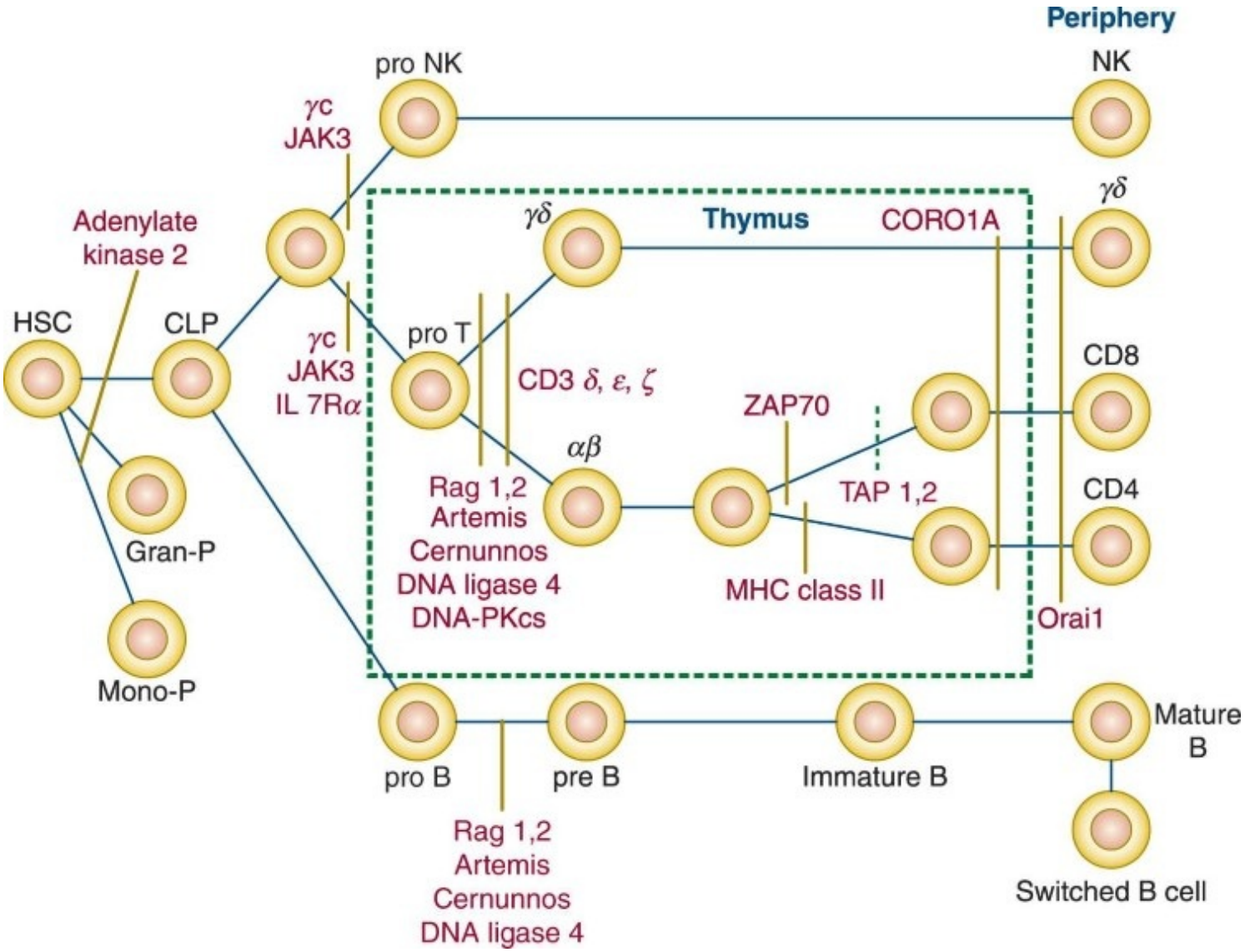


FIGURE 50–1 Disruption of the normal T-cell development by mutations of genes known to cause a severe combined immunodeficiency syndrome. (Source: *Williams Hematology,* 9th ed, Chap. 80, Fig. 80–1.)

Molecular Defects and Pathogenesis of SCID

Resulting from Increased Apoptosis of Lymphocyte Progenitors

- Adenosine deaminase (ADA) deficiency:
  — Inheritance is autosomal recessive.
  — Characteristics include the virtual absence of T lymphocytes as a consequence of absence of ADA. Resultant high intracellular levels of adenosine and deoxyadenosine and phosphorylated metabolites result in T-cell progenitor apoptosis.
  — A reduction in number of B cells also occurs.
- Purine nucleoside phosphorylase (PNP) deficiency
  — High levels of deoxyguanosine triphosphate cause destruction of immature thymocytes and neuronal toxicity.
  — Characteristics include decreased T-cell counts. B and NK cells are unaffected.
- Adenylate kinase 2 deficiency
  — The original term for this was *reticular dysgenesis*. It was so named because absence of cells in lymphohematopoietic organs left a background of reticular cells and fibers and the inference was drawn, erroneously, that this stroma could not support immune and blood cell development.
  — Autosomal recessive SCID is characterized by extreme lymphopenia, absence of neutrophils (loss of G-CSF responsiveness), and sensorineural deafness.
  — Severe sepsis occurs early in infancy.
  — Successful treatment with allogeneic hematopoietic stem cell transplantation indicates it is a cellular and not stromal (reticular cell) disorder.

Resulting from Defective Signaling Through the T-cell Receptor (TCR)

- Defects of interleukin (IL)-7 receptor(R)–mediated signaling abrogate development of T cells and defects in IL-15R signaling affect development of NK cells, resulting in SCID type T-B+NK-.
- X-linked *IL2R*γ gene mutations account for 40% of all SCID cases and result in lack of T and NK cells (T-B+NK-). However, B-cell function is impaired by failure of T helper function and a nonfunctional common gamma chain shared by several other key IL receptors (eg, 2R, 4R, 9R, 15R, and 21R).
- JAK3 deficiency is an autosomal recessive disorder with a phenotype identical to X-linked SCID type T-B+NK-.
- Defects in V(D) J recombination affect both T- and B-cell development and cause T-B-NK+ SCID.
- RAG1 and RAG2 deficiencies cause T-B-NK+ SCID and account for 1% of all SCID cases.
- Defects of the CD3, δ, ε, or ζ chains affect signaling through the TCR and cause T-B+NK+ SCID.

Clinical Features of SCID

- Characteristic features include *P jiroveci* pneumonia, cytomegalovirus (CMV), adenovirus, parainfluenza 2 virus, respiratory syncytial virus, chronic diarrhea, failure to thrive, and persistent candidiasis.
- Infections develop in first months of life.
- Lymphoid tissue is hypoplastic (atrophic tonsils and lymph nodes).

- Absence of thymic shadow is apparent on chest radiograph.

Laboratory Features of SCID

- Lymphocyte count is usually less than 2.0×10^9/L.
- In infants, there is marked blood T-cell deficiency. Blood CD3+ cells should be determined.
- Maternal T-cell engraftment and "leaky" SCID are characterized by expression of CD45RA+, whereas infant T cells are CD45RA naive and fail to respond to mitogens in vitro.
- Ig levels are low in infants.
- Eosinophilia and elevated levels of IgE both occur.
- Marrow abnormalities are observed in ADA, PNP, XLF, and LIG4 deficiencies.
- In ADA and PNP deficiency-related SCID, there are elevated levels of deoxyadenosine triphosphate, and deoxyguanosine triphosphate, respectively, in red cells.

Treatment, Course, and Prognosis of SCID

- This immunodeficiency is fatal if untreated.
- High-dose intravenous sulfamethoxazole/trimethoprim (20 mg/kg) is effective in treating *P jiroveci* pneumonia.
- CMV should be treated with ganciclovir.
- Adenoviral infections should be treated with cidofovir.
- IVIG and antimicrobial prophylaxis should be administered to reduce risk of infections.
- Survival is dependent on immune reconstitution by allogeneic hematopoietic stem cell transplantation.
- Enzyme replacement has benefited SCID patients with ADA deficiency and gene therapy has been successful in that variant of SCID without adverse events (eg, acute leukemia from insertional mutagenesis).

OTHER COMBINED IMMUNODEFICIENCIES

Omenn Syndrome

- Mutations in *RAG1* and *RAG2*, which restrict T-cell maturation and function, are the most common. For example, negative selection of autoreactive T cells is reduced and regulator T cell development is impaired.

Clinical Findings

- Severe infections occur.
- The syndrome is marked by early onset, with diffuse rash or generalized erythroderma.
- Alopecia may be present.
- There is lymphadenopathy and hepatosplenomegaly.
- A pattern of congenital anomalies occurs together more frequently than one would expect on the basis of chance.

Laboratory Findings

- Leukocytosis with eosinophilia is present.
- Hypoproteinemia with edema occurs.
- Oligoclonal expansion of anergic, activated T lymphocytes infiltrate and damage target tissues.
- Decreased serum IgG, M, and A levels occur, but IgE is elevated.
- Distribution of blood CD4 and CD8 lymphocyte subsets are skewed.
- Lymphocyte production of IL-4 and IL-5 is increased.
- Lymphocyte response to antigens nonexistent and response to mitogens is varied.
- Blood B and NK cell counts are abnormal in some patients.

Treatment

- The optimal approach is allogeneic hematopoietic stem cell transplantation.
- Patients require aggressive nutritional support, correction of hypoproteinemia, and treatment or prevention of infections with antibiotics, antifungals, and serum immunoglobulin replacement.
- Careful use of immunosuppression with glucocorticoids or cyclosporine A may mitigate T-cell induced tissue damage.

Zeta-Chain-Associated Protein Kinase (ZAP)-70 Deficiency

- This form of SCID results from the inability to support positive selection of CD8+ lymphocytes in the thymus.

Clinical Features

- Severe infections occur.
- Palpable lymph nodes and thymic shadow are visible on the chest radiograph.
- CD8+ T cells are reduced, but the absolute lymphocyte count is normal.
- Response to mitogens in vitro is reduced, indicating defective CD4+ lymphocytes.
- Some, but not all patients, have severe hypogammaglobulinemia.

Treatment

- Optimal approach is allogeneic hematopoietic stem cell transplantation.

Major Histocompatibility Complex Class I Deficiency

- Characterized by reduced expression of major histocompatibility complex (MHC) class I molecules at the cell surface.
- Inheritance is autosomal recessive. The cause may be mutations in *TAP1, TAP2,* or Tapasin genes, whose defects interfere with intracellular transport of antigens and their loading onto MHC class I molecules and cell surface expression of the MHC complex.

Clinical Findings

- Low levels of CB8+ T cells and Ig levels are variable.
- Recurrent respiratory infections occur in children.
- Chronic inflammatory lung disease and skin lesions are found in patients with *TAP1* and *TAP2*

deficiencies.
- Chronic lung disease is usually the cause of death.

Treatment
- Prophylactic and therapeutic measures used for cystic fibrosis are beneficial. Maintaining liquid pulmonary secretions and appropriate antibiotic use are important.

MHC Class II Deficiency
- Inheritance is autosomal recessive.
- Lack of MHC class II expression is characteristic.
- This is found in North African populations.
- Four gene mutations are known: *CIITA*, *RFXANK*, *RFX5*, and *RFXAP*.
- These mutations encode defective transcription factors that normally control MHC class II antigen expression by binding to the proximal promoters of the MHC class II gene.

Clinical Features
- Blood CD4+ T cell count is reduced.
- Severe lung infections are present.
- Chronic diarrhea occurs.
- Sclerosing cholangitis is found, often secondary to *Cryptosporidium* or CMV infection.

Treatment and Course
- Prognosis is poor.
- Respiratory infections are predominant cause of death.
- Nutritional support is often needed and antibiotic prophylaxis and immunoglobulin replacement therapy are required, but with marginal impact on long-term prognosis.
- Allogeneic hematopoietic stem cell transplantation may be necessary. Severe graft-versus-host disease is common.

Coronin-1A Deficiency
- This condition is characterized by mutations affecting both alleles of the *CORO1A* gene, an actin regulator expressed principally in hematopoietic cells.
- Such mutations regulate release of T lymphocytes from thymus and trafficking of naive T cells.
- Characteristic finding is T-cell lymphopenia with normal numbers of B and NK cells.
- Treatment by allogeneic hematopoietic stem cell transplantation is best approach if a donor available.

Signal Transducers and Activator 5b (STAT5b) Deficiency
- This autosomal recessive disease is characterized by the association of growth hormone insensitivity and a highly variable degree of immune deficiency.
- It results in impaired transcriptions of genes involved in immune system function and other non-immune related genes.

Clinical Features

- Short stature occurs in the face of normal or elevated levels of growth hormone but insulin growth factor is very low.
- Pulmonary infections, including *P jiroveci* pneumonia, are found.
- Patients have an increased susceptibility to severe viral diseases.
- Lung fibrosis occurs.
- There is a failure to respond to growth hormone replacement therapy.
- Glucocorticoids may be beneficial if there is evidence of lung fibrosis.

Ca^{2+} Entry Channel Deficiency

- This disorder is autosomal recessive.
- It results from mutations of both *ORAI1* and *STIM1* genes, the products of which regulate the function of calcium channels in the cell membrane and endoplasmic reticulum, respectively.
- Clinical features resemble SCID.
- Additional features include nonprogressive myopathy, ectodermal dysplasia, hepatosplenomegaly, hemolytic anemia, and thrombocytopenia.
- In spite of hypergammaglobulinemia, specific antibody responses are defective and T cells do not respond to mitogens in part as a result of failure of calcium influx after stimulation.
- Allogeneic hematopoietic stem cell transplantation can correct the defect.

DEFECTIVE THYMIC DEVELOPMENT

DiGeorge Syndrome

- This developmental disorder is caused by abnormal cephalic neural crest migration and differentiation in the third and fourth pharyngeal arches during embryonic development.
- Approximately 75% of persons with this phenotype have a deletion at band q11.2 on chromosome 22 (referred to as 22q11.2 deletion syndrome).

Clinical and Laboratory Features

- This mild to moderate immune deficiency involves T-cell maturation and function, with T-cell numbers often less than 1.5×10^9/L.
- Phenotype varies but classically has the triad of (1) congenital cardiac defects, (2) hypocalcemia as a result of parathyroid insufficiency, and (3) immune deficiency as a consequence of aplasia or hypoplasia of the thymus.
- Fifty percent to 80% of patients develop cardiac defects, and 50% to 60% develop hypocalcemia.
- The incidence of autoimmune diseases, such as rheumatoid arthritis and thyroiditis, is high.
- As young adults, social, behavioral, and psychiatric problems develop.
- Patients with profound T-cell deficiency may develop B-cell lymphomas.
- If suspected, fluorescence in situ hybridization can be used to detect 22q11.2 deletion.

Treatment

- Cardiac defects and hypocalcemia require immediate attention.
- Patients may require antibiotic prophylaxis, IVIG therapy, and if T-cell function is absent, immune reconstitution by allogeneic hematopoietic stem cell transplantation if a donor is available.

PRIMARY IMMUNODEFICIENCY DISORDERS PRESENTING AS AUTOIMMUNE DISEASES

- The concept of a link between immune dysregulation and autoimmunity has been strengthened by the discovery of distinct single gene defects resulting in unusual susceptibility to autoimmune diseases.

IPEX Syndrome

- The acronym IPEX derives from the presence of **i**mmune dysregulation, **p**olyendocrinopathy, **e**nteropathy, and an X-linked inheritance.
- The syndrome is characterized by early-onset diarrhea secondary to autoimmune enteropathy.
- Multiple endocrinopathies, including type 1 diabetes mellitus, thyroiditis, and, rarely, adrenal insufficiency, are present.
- Autoimmune hemolytic anemia, thrombocytopenia, and neutropenia are common complications.
- Eczema or other chronic dermatitis may occur.
- There is elevated serum IgA and IgE, with absence of CD4+CD25+FOXP3+regulatory T cells.
- Cyclosporine A, tacrolimus, sirolimus, or glucocorticoids can provide temporary amelioration.

APECED Syndrome

- The acronym APECED derives from the presence of **a**utoimmune **p**olyendocrinopathy, **c**andidiasis, and **e**ctodermal **d**ystrophy.
- This autosomal recessive disorder is also known as autoimmune polyglandular syndrome (APS) type I.
- It results from mutations in the *AIRE* gene, which causes a decrease in the expression of tissue restricted antigens, failure of negative selection of autoreactive T cells in the thymus, and a resultant release of autoreactive T cell clones to peripheral lymphatic tissues.
- Isolated populations, such as Finns, Iranian Jews, and Sardinians, are affected.
- Patients present with chronic mucocutaneous candidiasis and endocrinopathies, predominantly involving the parathyroid and adrenal glands, less frequently the thyroid and the pancreas.
- It is associated with ectodermal manifestations, such as dystrophic finger nails and dental enamel.

Autoimmune Lymphoproliferative Syndrome

- The acronym ALPS derives from the **a**utoimmune **l**ympho**p**roliferative **s**yndrome.

- Mutations are found in the genes required for "programmed cell death." Fas-mediated apoptosis pathway mutations account for about 85% of cases:
  — Mutations in CD95 (ALPS type Ia)
  — Mutations in CD95L (ALPS type Ib)
  — Mutations in caspase 10 or caspase 8 (ALPS type II) (approximately 5% of cases)
  — No mutations of Fas, FasL, or caspases (ALPS type III) (approximately 10% of cases)
- The phenotype is caused by defective apoptosis of lymphocytes, resulting in polyclonal lymphadenopathy, hepatosplenomegaly, and autoimmune disorders, which most commonly include autoimmune hemolytic anemia, thrombocytopenia, and neutropenia.
- Spleen and lymph nodes show pronounced hyperplasia and the T-cell population includes a large proportion of TCRα/β+ CD4-CD8- cells.
- Treatment options include immunosuppressive therapy.
- Splenectomy is recommended in patients with large spleens.
- Long-term prognosis is poor.
- Lymphoma develops in about one of ten patients.

OTHER WELL-DEFINED IMMUNODEFICIENCY DISEASES

Wiskott-Aldrich Syndrome

Definition

- Wiskott-Aldrich syndrome (WAS) is an X-linked disorder characterized by thrombocytopenia, small platelets, eczema, recurrent infections, immunodeficiency, and a high incidence of autoimmune diseases and malignancies.
- Phenotype is associated with null-mutations of the gene that encodes the WAS protein.
- Milder phenotype is called X-linked thrombocytopenia (XLT).
- Mild eczema and few problems are characteristic.

Clinical and Laboratory Features

- Thrombocytopenia is in the range of 20 to 60 × 10^9/L and microplatelets, but numbers of marrow megakaryocytes are normal.
- Hemorrhagic manifestations may be mild.
- Classic WAS is characterized by bacterial, fungal, and viral infections.

Treatment

- Was may require antibiotic prophylaxis and IVIG.
- If autoimmune symptoms arise, immunosuppressive therapy may be required.
- Early allogeneic hematopoietic stem cell transplantation is treatment of choice; outcome is excellent if a matched-related or unrelated donor can be identified.
- Splenectomy ameliorates the bleeding tendency by increasing the number of blood platelets.
- XLT patients often have an excellent prognosis but may develop complications, including serious bleeding, autoimmune diseases, and malignancies.
- Standard-conditioning for myeloablative transplantation (busulfan, cyclophosphamide, with or

without antithymocyte globulin) is required.

Hyperimmunoglobulin E Syndromes

Autosomal Dominant Hyper-IgE Syndrome

- This is an autosomal dominant or sporadic multisystem immunodeficiency.
- Eczema, *S aureus*-induced skin abscesses, recurrent pneumonia with abscess and pneumatocele formation, *Candida* infections, and skeletal and connective tissue abnormalities are characteristic.
- Serum IgE levels are greater than 2000 IU/mL and often much greater.
- Features include eosinophilia, neutrophil chemotactic defects, and decreased lymphocyte proliferation to specific antigens.
- Treatment includes prophylactic antibiotic therapy to decrease the frequency of *S aureus* pulmonary infections.
- Antifungal therapy is indicated to prevent recurrent *Candida* infections.
- Allogeneic hematopoietic stem cell transplantation has had variable benefits.

Autosomal Recessive Hyper-IgE Syndromes

- These disorders are characterized by elevated serum levels of IgE, as well as recurrent bacterial, fungal, and viral infections, including herpes simplex, therapy-resistant molluscum contagiosum, and recurrent varicella zoster.

Immuno-osseous Dysplasias

Cartilage-Hair Hypoplasia

- This autosomal recessive disorder is characterized by short-limbed dwarfism, light-colored hypoplastic hair, marrow cell dysplasia, Hirschsprung disease, a variable degree of immunodeficiency from normal to severe, and increased susceptibility to malignancies.
- In the severe cases, allogeneic hematopoietic stem cell can correct the immune abnormalities.

Schimke Syndrome

- The disease is caused by a gene that encodes for a chromatin remodeling protein.
- This autosomal recessive condition is characterized by dwarfism, microcephaly, cognitive and motor abnormalities, renal impairment leading to renal failure, facial dimorphisms, marrow failure, premature atherosclerosis, and immunodeficiency ranging from T-cell lymphopenia to SCID.
- Recurrent bacterial, fungal, and viral infections, including with opportunistic organisms, occur in more than 50% of patients.
- Combined allogeneic hematopoietic stem cell and renal transplantation has been used with success.

Warts, Hypogammaglobulinemia, Infections, and Myelokathexis Syndrome

- This autosomal dominant disorder is caused by a mutation in the *CXCL4* gene that disrupts the

chemokine CXCL12 receptor involved in leukocyte trafficking.
- Retention and apoptosis of neutrophils in marrow (myelokathexis) causes severe neutropenia.
- Early onset of recurrent bacterial infections is common.
- Warts resulting from papillomavirus infection develop in second decade of life.
- Hypogammaglobulinemia, lymphopenia, and low B-cell counts are common.
- IVIG replacement therapy and antibiotics are given as required. Recombinant G-CSF can increase neutrophil count.
- Warts are resistant to local therapy and should be monitored for neoplastic transformation.

CHROMOSOMAL INSTABILITY SYNDROMES ASSOCIATED WITH IMMUNODEFICIENCY

- These syndromes are characterized by increased spontaneous or induced DNA breaks, susceptibility to infections secondary to immune deficiency, and an increased risk of malignancies.
- Genes responsible for these diseases protect human genome integrity by contributing to the complex task of double-strand break repair.

Ataxia-Telangiectasia

- This multisystem disorder is characterized by immunodeficiency, progressive neurologic impairment, and ocular and cutaneous telangiectasia.
- Immune deficiency is variable and may include cellular and humoral immunity.
- Thymus is small.
- Mutation in *ATM* gene results in inability to repair double-stranded DNA breaks.
- Common to have recurrent respiratory infections that result in chronic lung disease.
- Phenotype is low or absent IgA and IgE and is often combined with IgG_2 and IgG_4 deficiency.
- Clinical manifestations include cerebellar ataxia, which becomes evident when a child begins to walk. Involuntary movements become a handicap and most are wheelchair bound by age 10 years.
- Children do not develop normal speech patterns.
- Cortical cerebellar degeneration involves primarily Purkinje and granular cells; progressive changes to the central nervous system also occur.
- Cytogenetic abnormalities include chromosomal breaks, translocations, rearrangements, and inversions; these defects increase following in vitro exposure of cells to radiation.
- Elevation of serum α-fetoprotein is a very common and characteristic laboratory finding.
- Infections and cancer (lymphomas, usually T-cell type [50%], leukemias [25%], and solid tumors [25%]) are the most common causes of death.

Ataxia-Telangiectasia–Like Disorder

- Clinical features are similar to those of ataxia-telangiectasia with progressive ataxia but a slower progression of disease.
- Mutations in the gene encoding the hMre11 protein, part of the DNA repair complex, are characteristic.

Nijmegen Breakage Syndrome

- This syndrome is characterized by short stature, microcephaly, a bird-like face, immunodeficiency, chromosomal instability, increased sensitivity to radiation and radiomimetic drugs (eg, alkylating agents), and a high likelihood of developing malignancies.
- There is absence of telangiectasia formation and of neurodegeneration.
- Development of respiratory infections is common.
- Humoral and cellular immunity is defective.
- Characteristics include increased chromatid and chromosome breaks, rearrangements/translocations involving chromosomes 7 and 14, telomere fusions, radioresistant DNA synthesis, and hypersensitivity to radiation.
- Incidence of lymphoid malignancies and certain solid tumors (eg, rhabdomyosarcoma) is high.
- Prophylactic antibiotics and IVIG are useful for patients with recurrent infections.
- Allogeneic hematopoietic stem cell transplantation has been successful.

Bloom Syndrome

- This syndrome is caused by mutation in *BMS* gene that encodes a protein involved in sensing DNA damage and contributes to maintenance of genomic integrity during DNA replication or repair.
- Characteristics include short stature, hypersensitivity to sunlight, increased susceptibility to infections, and a predisposition to early development of cancer (eg, lymphoma and leukemia in first two decades of life) and cancer of colon, skin, and breast at a later age.
- Fifty percent of patients develop cancer before the age of 25 years.
- It is confirmed by demonstrating excessive numbers of sister-chromatid exchanges, increased chromatid gaps and breaks, and the presence of quadriradial configuration composed of two homologous chromosomes.
- Patients may benefit from antibiotic prophylaxis and IVIG therapy, if immune deficiency is documented. Because of increased radiation sensitivity, exposure to any form of irradiation should be restricted.

CYTOTOXICITY DISORDERS

Familial Hemophagocytic Lymphohistiocytosis

- Familial hemophagocytic lymphohistiocytosis (FHL) is characterized by uncontrolled proliferation of activated lymphocytes and histiocytes that secrete large amounts of proinflammatory cytokines (see Chap. 36).

Clinical and Laboratory Features

- Signs and symptoms appear within first year of life.
- Symptoms include high fever, severe hepatosplenomegaly, lymphadenopathy, hemorrhagic manifestations from thrombocytopenia, and edema. Also, patient may have neurologic symptoms.
- Immunologic findings include persistently impaired cytolytic activity of NK cells and elevated levels of inflammatory cytokines (interferon [IFN]-γ, IL-1, IL-6, tumor necrosis factor [TNF]-

γ) in the blood.
- The diagnosis of FHL forms that are characterized by reduced NK cell degranulation (such as *UNC13D* and *STX11* defects) may be facilitated by the analysis of membrane expression of the lysosomal marker CD107a.
- Without treatment, FHL is fatal.
- If disease is active, treatments include antimicrobials, etoposide, immune suppression (antithymocyte globulin), cyclosporine A, and dexamethasone.
- Cure can be achieved by allogeneic hematopoietic stem cell transplantation.

X-Linked Lymphoproliferative Disease (XLP1 and XLP2)

- XLP1 is characterized by mutations in the *SH2D1A* gene that encodes the SLAM-associated protein (SAP) involved in T and NK cell signaling and impair T and NK cell cytotoxicity capability.
- XLP2 is characterized by mutations in the *XIAP* gene and also lack of NK and T cells.

Clinical and Laboratory Features

- Fulminant Epstein-Barr virus (EBV) infectious mononucleosis occurs in 60% of cases.
- Hypogammaglobulinemia can follow primary EBV infection.
- EBV-related lymphoma in 30% of cases (especially Burkitt lymphoma).
- Flow cytometry can be used to detect lack of SAP protein expression in circulating T and NK lymphocytes.

Treatment and Prognosis

- If untreated, approximately 70% of patients die within 10 years of onset.
- Mortality rate is higher (approaches 100%) in patients who have fulminant infectious mononucleosis.
- Treatment of choice is allogeneic hematopoietic stem cell transplantation when performed early in life before EBV infection.
- Nonmyeloablative allogeneic hematopoietic stem cell transplantation may be useful after EBV infection supervenes in patients with severe organ toxicity.
- Use of anti-CD20 monoclonal antibody can reduce EBV load and improve clinical status.
- Igs can be used to reduce the risk of infections in patients with hypogammaglobulinemia.
- Anti-TNF-α therapy or etoposide may be useful in patients with active EBV infection and a severe systemic inflammatory response.

Chédiak-Higashi Syndrome

- This autosomal recessive disorder is characterized by immune dysregulation with impaired cellular cytotoxicity, partial oculocutaneous albinism, platelet functional abnormalities, and neurologic involvement (see Chap. 33).
- It is caused by mutations in the *LYST* gene, the encoded protein of which contributes to sorting lysosomal proteins and fusing lysosomal vesicles.
- Common features include bruises and bacterial and viral infections.
- “Accelerated phase” of syndrome is characterized by high fever, hepatosplenomegaly,

coagulation abnormalities, increase of liver enzymes and bilirubin (with possible jaundice), edema, and neurologic symptoms.
- Morphologic hallmark of disease is abnormally large granules in lymphocytes, neutrophils, platelets, melanocytes, and neurons.
- Examination of the blood film permits detection of the enlarged and abnormal granules in blood cells.
- Allogeneic hematopoietic stem cell transplantation can ameliorate immune and cellular abnormalities but does not prevent progressive neurologic involvement.

Griscelli Syndrome Type 2

- This autosomal recessive syndrome is characterized by immunodeficiency and hypopigmentation. A variable degree of neurologic involvement is also present.
- It is caused by mutations in the *RAB27A* gene, which encodes a guanosine triphosphate involved in intracellular transport of granules.
- Patients are susceptible to pyogenic infections.
- "Accelerated phase" of syndrome is characterized by high fever, hepatosplenomegaly, neutropenia, and thrombocytopenia.
- Hypopigmentation is a result of large clumps of melanin in the hair shafts.
- Allogeneic hematopoietic stem cell transplantation can ameliorate the manifestations of the disorder.

Hermansky-Pudlak Type 2

- This disorder is caused by mutations of the *AP3B1* gene, which encodes a protein that regulates sorting of lysosomal membrane proteins to the granules.
- Characteristics include oculocutaneous albinism, bleeding tendency, recurrent infections, and moderate to severe neutropenia.
- The bleeding tendency results from decreased platelet dense granules and faulty degranulation.
- Missorting of tyrosinase in melanocytes accounts for albinism.
- Bony anomalies (with dysplastic acetabulae), facial dysmorphism, and development of pulmonary fibrosis are features.
- Control of infections is important to manage disease.
- G-CSF may improve chronic neutropenia.

IMMUNODEFICIENCIES WITH SELECTIVE SUSCEPTIBILITY TO PATHOGENS

Toll-Like Receptor (TLR)-Signaling Defects

- Deficiencies of IL-1 receptor-associated kinase (IRAK)-4, myeloid differentiation factor 88 (MyD88), TLR3, and UNC-93B proteins occur.
- Two phenotypes have been identified.
- TLR-signaling defects with increased susceptibility to herpes simplex virus encephalitis are associated with mutations in the UNC-93B1 gene.
- TLR-signaling defects with increased susceptibility to recurrent, invasive pyogenic infection

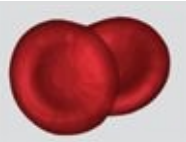
are associated with mutations in IRAK-4 and MyD88.
- Diagnosis can be suspected based on the history of infection associated with poor inflammatory responses.
- Defects may involve other microbial pattern-recognition signaling pathways resulting in increased susceptibility to fungal infections.

Mendelian Susceptibility to Mycobacterial Disease

- Defects occur along the JAK-STAT4 pathway.
- IL-12p40 deficiency is characterized increased risk of severe infections due to Calmette-Guerin and environmental mycobacteria.
  — This is the only genetically determined cytokine deficiency known in humans
  — Treatment is with antibiotics and IFN-γ
- IL-12Rβ_1 deficiency is characterized by infections with mycobacteria of low virulence and *Salmonella* species.
  — Treatment with antibiotics and IFN-γ is effective and prognosis is good.
- IFN- γR1 and IFN- γR2 deficiencies produce variable susceptibilities.
  — Persons with complete deficiencies develop severe infections with mycobacteria early in life with lack of granuloma formation.
  — Complete STAT1 deficiency causes increased susceptibility to mycobacterial disease with a severe clinical course.
  — Dominant partial STAT1 deficiency is caused by a heterozygous mutation that allows formation of the IFN-α/β-dependent ISGF3 transcription factor, but abrogates expression of the γ-activating factor, composed of STAT1 homodimers. Affected individuals have either a mild clinical course, characterized by selective susceptibility to mycobacterial infections or are asymptomatic.

GENETICALLY DETERMINED DEFICIENCIES OF THE COMPLEMENT (C) SYSTEM

- Mutations in the classic pathways (C1q, C1r/C1s, C4, C2, and C3) result in pyogenic infections and autoimmune diseases.
- Mutations in the alternative pathway (factors B, D, h, properdin) result in meningococcal and pneumococcal sepsis.
- Mutations in the terminal complement components (C5-C9) result in increased susceptibility to *Neisseria* species infections.
- Mutations in C1 esterase inhibitor (*C1-INH*) gene is the cause of hereditary angioedema.
- Diagnosis of a deficiency assesses the hemolytic function of CH50 and AH50.
  — If CH50 is absent and AH50 is normal, there may be a C1, C4, or C2 defect.
  — If CH50 is normal and AH50 is absent, there may be a properdin or factor D defect.
  — If CH50 and AH50 are abnormal, there may be a C3 to C8 defect.
  — CH50 is usually at half normal in the case of C9 defects.
- Treatment is determined by the type of deficiency.



For a more detailed discussion, see Hans D. Ochs and Luigi D. Notarangelo: Immunodeficiency Diseases, Chap. 80, in *Williams Hematology*, 9th ed.

CHAPTER 51

Hematological Manifestations of the Acquired Immunodeficiency Syndrome

DEFINITION AND HISTORY

- Patients with serologic evidence of infection with the human immunodeficiency virus (HIV) can be diagnosed as having acquired immunodeficiency syndrome (AIDS) based on "AIDS-defining conditions" (Table 51–1).
- Patients with HIV are living longer in the era of highly active antiretroviral therapy (HAART).
- The United Nations estimated that 35 million people worldwide were living with HIV infection in 2012, with the majority being infected by heterosexual contact.

TABLE 51–1 AIDS-DEFINING CONDITIONS

Bacterial infections, multiple or recurrent*
Candidiasis of bronchi, trachea, or lungs
Candidiasis of esophagus†
Cervical cancer, invasive§
Coccidioidomycosis, disseminated or extrapulmonary
Cryptococcosis, extrapulmonary
Cryptosporidiosis, chronic intestinal (> 1 month's duration)
Cytomegalovirus disease (other than liver, spleen, or nodes), onset at age > 1 month
Cytomegalovirus retinitis (with loss of vision)†
Encephalopathy, HIV realted
Herpes simplex: chronic ulcers (> 1 month's duration) or bronchitis, pneumonitis, or esophagitis (onset at age > 1 month)
Histoplasmosis, disseminated or extrapulmonary
Isosporiasis, chronic intestinal (> 1 month's duration)
Kaposi sarcoma†
Lymphoid interstitial pneumonia or pulmonary lymphoid hyperplasia complex*†
Lymphoma, Burkitt (or equivalent term)
Lymphoma, immunoblastic (or equivalent term)
Lymphoma, primary, of brain
Mycobacterium avium complex or *Mycobacterium kansasii*, disseminated or extrapulmonary†
Mycobacterium tuberculosis of any site, pulmonary,†§ disseminated,† or extrapulmonary†
Mycobacterium, other species or unidentified species, disseminated† or extrapulmonary†
Pneumocystis jiroveci pneumonia†
Pneumonia, recurrent†§
Progressive multifocal leukoencephalopathy
Salmonella septicemia, recurrent
Toxoplasmosis of brain, onset at age > 1 month†
Wasting syndrome attributed to HIV

AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus.
*Only among children younger than age 13 years. (Centers for Disease Control and Prevention. 1994 Revised classification system for human immunodeficiency virus infection in children less than 13 years of age. *MMWR Morb Mortal Wkly Rep*.

1994;43(RR-12). Available at http://www.cdc.gov/mmwr/PDF/rr/rr4312.pdf.)

[†]Condition that might be diagnosed presumptively.

[§]Only among adults and adolescents older than age 13 years. (Centers for Disease Control and Prevention. 1993 Revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *MMWR Morb Mortal Wkly Rep*. 1992;41(RR-17):1-19.)

Data from Centers for Disease Control and Prevention: 1994 Revised classification system for human immunodeficiency virus infection in children less than 13 years of age. *MMWR Morb Mortal Wkly Rep*. 1994;43(RR-12). Available at http://www.cdc.gov/mmwr/PDF/rr/rr4312.pdf, and 1993 Revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *MMWR Morb Mortal Wkly Rep*. 1992;41(RR-17):1-19.

Source: *Williams Hematology,* 9th ed, Chap. 81, Table 81–2.

ETIOLOGY AND PATHOGENESIS

Human Immunodeficiency Virus 1

- The primary cause of AIDS is infection with HIV-1.
- HIV-1 is a member of the Lentivirinae subfamily of retroviruses.
  - — Retroviruses are RNA viruses that induce a chronic cellular infection by converting their RNA genome into a DNA provirus that is integrated into the genome of the infected cell.
- Infection by these lentiviruses is characterized by long periods of clinical latency followed by gradual onset of disease-related symptoms.

Transmission of HIV

- The four main routes of HIV infection are:
  - — *Sexual contact* with an infected partner
    - The risk for HIV transmission through sexual contact may be increased in persons with other concurrent sexually transmitted diseases.
  - — *Parenteral drug use*
    - Sharing needles and syringes is the main mode of transmission.
  - — *Exposure to infected blood or blood products*
    - Ninety percent of those who receive a contaminated unit of blood become infected.
    - Risk of HIV transmission through transfusion of a unit of red blood cells tested negative for antibodies to HIV is approximately 1 in 493,000 transfusions.
  - — *Perinatal exposure*
    - HIV-1 may be transmitted in utero; intrapartum (at the time of delivery); or postpartum, through ingestion of HIV-1-infected mother's milk.
    - The risk of infection from mother to infant differs in various parts of the world, ranging from approximately 15% in Europe and approximately 40% to 50% in Africa.
    - The risk of perinatal transmission is increased in mothers with more advanced HIV disease, higher HIV-1 viral load in the plasma, or a history of cigarette smoking and/or active drug abuse.
    - Antiretroviral agents in pregnancy and delivery, with subsequent administration to the infant for the first 6 weeks of life, has resulted in a dramatically reduced rate of transmission, from approximately 25% to 8% with zidovudine alone and even lower with use of HAART.

Pathogenesis of HIV Infection

- HIV infection results in aberrant immune regulation and immunodeficiency.
  — Defects include decreased lymphocyte proliferative response to soluble antigens in vitro, decreased helper response in immunoglobulin (Ig) synthesis, impaired delayed hypersensitivity, decreased interferon (IFN)-γ production, and decreased helper T-cell response of virally infected cells.
- Infection with HIV-1 results in a progressive loss of CD4+ T lymphocytes.
- Monocytes, macrophages, and follicular dendritic cells of the lymph nodes express CD4 antigen and can be infected by HIV.
- Macrophage-tropic (M-tropic) strains of HIV use the CCR5 chemokine receptor to infect both macrophages and CD4+ lymphocytes.
- Loss of follicular dendritic cells results in defective antigen processing in patients with advanced HIV disease.
- Pronounced polyclonal activation of B lymphocytes is common, especially during early stages of disease and results in hypergammaglobulinemia.
- Antigen-specific B-cell proliferation and antibody production are decreased in patients with AIDS.
- HIV infection is associated with an increase in autoimmune phenomena and an increase risk of B-cell lymphomas.
- Natural killer (NK) cell activity is decreased in the blood of HIV-infected individuals.

DIAGNOSIS OF HIV

- The primary diagnostic screening test is the enzyme-linked immunosorbent assay, or ELISA, for detection of antibodies to HIV glycoproteins.
  — The median time from initial infection to first detection of HIV antibody is about 2 to 4 weeks.
- Testing by polymerase chain reaction (PCR) may detect the presence of HIV within 1 week of initial infection.

COURSE AND PROGNOSIS

- Infection by HIV-1 causes a gradual but progressive loss of immune function, leading to development of nonspecific symptoms and then specific infections and/or neoplastic disease.
  — Without effective antiretroviral therapy, patients who develop AIDS generally experience relentless deterioration in physical health and ultimately die of one or more complications secondary to acquired immunodeficiency, organ dysfunction, and/or malignancy associated with HIV infection.
- **Table 51–2** lists examples of common opportunistic infections by CD4 count.
- **Table 51–3** provides an overview of primary prophylaxis for the most common opportunistic infections.
- Acute retroviral syndrome
  — An acute clinical illness often is associated with initial HIV infection.

— This acute phase occurs in approximately 75% of patients.
— It typically begins 1 to 3 weeks after primary infection and lasts for 1 to 2 weeks.
— Symptoms include fatigue, malaise, headache, fever, rash, and photophobia lasting several weeks; there is myalgia and a morbilliform rash.
— Generalized lymphadenopathy termed *persistent generalized lymphadenopathy* may occur toward the end of the acute retroviral syndrome and persist indefinitely.

- Early asymptomatic HIV disease
  — After resolution of the acute retroviral syndrome, the patient usually returns to a state of well-being.
- Advanced symptomatic HIV disease can define the diagnosis of AIDS.
- The list of AIDS-defining clinical conditions is presented in **Table 51–1**.
- Laboratory features of disease progression
  — Quantitation of plasma HIV RNA (viral load) and CD4+ lymphocyte count are the most useful parameters.

TABLE 51–2 EXAMPLES OF COMMON OPPORTUNISTIC INFECTIONS BY CD4 COUNT

CD4 Count	Opportunistic Infection or Condition
≥ 500 cells/μL	Any condition that can occur in HIV-uninfected persons, eg, bacterial pneumonia, tuberculosis, varicella-zoster, herpes simplex virus
350–499 cells/μL	Trush, seborrheic dermatitis, oral hairy leukoplakia, mulluscum contagiosum
200–349 cells/μL	Kaposi sarcoma, lymphoma
100–199 cells/μL	Pneumocystis pneumonia, *Candida* esophagitis, cryptococcal meningitis
< 100 cells/μL	*Toxoplasma* encephalitis, disseminated *Mycobacterium avium* complex, progressive multifocal leukoencephalophaty, cytomegalovirus retinitis, primary central nervous system lymphoma, microsporidia

HIV, human immunodeficiency virus.
Source: *Williams Hematology,* 9th ed, Chap. 81, Table 81–3.

TABLE 51–3 PRIMARY PROPHYLAXIS

Infection	Criteria	Treatment
Penumocystis pneumonia	CD4 < 200 cell/μL or <14% or oral candidiasis or an AIDS-defining illness	Trimethoprim-sulfamethoxazole or dapsone or aerosolized pentamidine
Tuberculosis	Purified protein derivative >5 m or + interferon-γ release assay	Isoniazid (INH) + pyridoxine
Toxoplasmosis	Immunoglobulin G+ and CD4 <100 cells/μL	Trimethoprim-sulfamethoxazole or dapsone+ primethamine+ leucovorin
Mycobacterium avium complex	CD4 <50 cells/μL	Azithromycin or clarithromycin

AIDS, acquired immunodeficiency syndrome.
Source: *Williams Hematology,* 9th ed, Chap. 81, Table 81–4.

HEMATOLOGIC ABNORMALITIES

Anemia

- Anemia is common in HIV-infected individuals, occurring in approximately 10% to 20% at initial presentation and diagnosed in approximately 70% to 80% of patients over the course of disease.
- Numerous causes for anemia exist in HIV-infected patients (**Table 51–4**).
- Anemia with a hemoglobin of less than 10 g/dL may be associated with shorter survival.
- Recovery from anemia is independently associated with improved survival.
- HAART can correct or improve anemia associated with HIV infection.
- Erythropoietin can correct or improve anemia associated with HIV infection.
  - — Low erythropoietin levels and blunted response to erythropoietin are extremely common in the setting of HIV infection.
  - — Erythropoietin 100 to 200 U/kg weight can be administered subcutaneously three times per week until improvement of the hemoglobin concentration and then approximately once every week or every other week to maintain a hemoglobin concentration of approximately 11 to 12 g/dL.
  - — Clinical trials have demonstrated the equivalent efficacy of 40,000 U of erythropoietin given weekly compared with the original thrice-weekly schedule in anemic HIV-infected patients.
  - — Patients with a baseline endogenous erythropoietin level of 500 IU/L or less more likely to respond to erythropoietin therapy.

TABLE 51–4 CAUSES OF ANEMIA IN HUMAN IMMUNODEFICIENCY VIRUS (HIV)

DECREASED PRODUCTION
HIV effect on hematopoiesis
Marrow infiltration (eg, *Mycobacterium avium* complex, histoplasmosis, non-Hodgkin lymphoma, Hodgkin lymphoma)
Pure red cell aplasia (Parvovirus B 19)
Drug suppression of hematopoiesis (eg, zidovudine)
Nutritional deficiency (eg, vitamin B_{12}, folate, iron)
Inflammation
INCREASED DESTRUCTION
Thrombotic thrombocytopenic purpura
Immunohemolytic anemia
Glucose-6-phosphate dehydrogenase deficiency (eg, dapsone, trimethoprim-sulfamethoxazole)
Hemophagocytic syndrome
LOSS
Gastrointestinal bleeding (eg, Kaposi sarcoma in gastrointestinal tract)

Source: *Williams Hematology,* 9th ed, Chap. 81, Table 81–9.

Neutropenia

- Neutropenia is reported in approximately 10% of patients with early, asymptomatic HIV infection and in more than 50% of individuals with more advanced HIV-related immunodeficiency.
  - — Thus, the risk of bacterial infection increased 2.3-fold for HIV-infected individuals with absolute neutrophil counts (ANC) less than 1.0×10^9 cells/L and increased by 7.9-fold in those with ANC levels less than 500 cells/µL.
- The use of HAART can be associated with improvement of neutropenia.

Thrombocytopenia

- Thrombocytopenia (< 100×10^9/L) is relatively common during the course of HIV infection.
  — The incidence of thrombocytopenia over 1 year was 9% in patients with clinical AIDS, 3% in patients with immunologic AIDS (< 200 CD4+ cells/μL), and approximately 2% in patients with neither clinical nor immunologic AIDS.
  — Thrombocytopenia is associated with history of:
    - AIDS
    - Injection drug use
    - History of anemia or lymphoma
    - African descent
- Thrombocytopenia is associated with shorter survival.
- Persons with HIV infection have a high risk of secondary thrombocytopenia because of increased risk of other infections or treatment with myelosuppressive medicine.
- What has previously been described as HIV-associated immune thrombocytopenic purpura (ITP) is increasingly characterized as "primary HIV-associated thrombocytopenia (PHAT)."
- In contrast to de novo ITP, PHAT is associated with a higher rate of splenomegaly, typically less severe thrombocytopenia, and a 20% spontaneous remission rate.
- Presence of platelet-specific antibodies, against both glycoprotein (GP) IIb and GPIIIa, have been detected in patients with PHAT.
- Antibodies against platelet GPIIb-IIIa have been demonstrated to be cross-reactive with HIV GP160/120.
- A further mechanism of antibody-induced destruction of platelets arises from the absorption of immune complexes against HIV.
- Mean platelet survival is decreased in patients with PHAT.
- Mean platelet production is decreased in patients with untreated PHAT.
- Reduced production of platelets in the setting of HIV infection may be direct due to infection of the megakaryocyte by HIV.
- The diagnosis of PHAT is clinical and requires the exclusion of secondary causes of thrombocytopenia and discontinuation of potentially myelosuppressive medications.
- Zidovudine may be effective in the treatment of patients with PHAT.
- HAART is effective for treatment of PHAT.
  — HAART was associated with a significantly increased platelet count after 3 months.
- Treatment with interferon-alpha (IFN-α) at 3,000,000 U given subcutaneously three times per week increases platelet counts after 3 weeks.
  — Platelet response was documented in 66%, with a mean increase of 60×10^9/L.
  — IFN-α was found to prolong platelet survival, whereas no significant increase in platelet production was noted.
- Treatment with high-dose intravenous immunoglobulin (IVIG) at 1000 to 2000 mg/kg may result in a significant rise in platelet counts within 24 to 72 hours.
  — IVIG often is reserved for use in patients who are acutely bleeding or require an immediate increase in platelet count.
- Use of anti-Rh immunoglobulin in nonsplenectomized Rh-positive patients with PHAT is another potential mode of therapy.
  — Requirements for effective therapy with anti-Rh (D) include presence of Rh+ red cells in

the patient, a baseline hemoglobin level adequate to permit a 1- to 2-g decrease as a result of hemolysis, and presence of a spleen.
  - — Patients were treated with 25 mg/kg intravenously over 30 minutes on two consecutive days.
  - — Patients responded with a platelet count greater than 50×10^9/L with response first noted at approximately 4 days and median response duration of 13 days.
  - — Maintenance therapy with anti-Rh immunoglobulin of 13 to 25 mg/kg intravenously administered every 2 to 4 weeks, resulted in a long-term response (> 6 months) in 70% of patients.
  - — Subclinical hemolysis due to the anti-Rh immunoglobulin occurred in all patients, with a decrease in hemoglobin of 0.4 to 2.2 g.
- Splenectomy has been used effectively to treat patients with intractable thrombocytopenia.
  - — A complete response was seen in 92% of patients (platelet count > 100×10^9/L).
  - — No difference was found when the survival or rate of progression to AIDS in the 68 splenectomized patients was compared with the rate in the 117 patients who did not undergo the procedure, indicating that splenectomy was not associated with more rapid progression of HIV disease.
  - — Approximately 6% of patients who underwent splenectomy in one series experienced fulminant infection.
- Prednisone at an oral dosage of 1 mg/kg per day has been associated with an 80% to 90% response rate in HIV-infected patients with thrombocytopenia secondary to intractable thrombocytopenia.
  - — The potential development of fulminant Kaposi sarcoma in dually HIV-infected and human herpes virus (HHV)-8–infected patients after use of glucocorticoids has dampened enthusiasm for the use of prednisone to treat thrombocytopenia in HIV-infected patients.

Pancytopenia

- **Table 51–5** shows the most common causes of pancytopenia in HIV infection.

TABLE 51–5 PANCYTOPENIA IN HUMAN IMMUNODEFICIENCY VIRUS (HIV)

- Advanced HIV with high viral load
- Medication side effect
- Malignancy in the marrow
- Non-Hodgkin lymphoma, Hodgkin lymphoma
- Infection in the marrow
- *Mycobacterium avium complex*, histoplasmosis, cytomegalovirus, *Mycobacterium tuberculosis*
- Castleman disease
- Hemophagocytic syndrome
- Alcohol abuse
- Vitamin B_{12} or folate deficiency

Source: *Williams Hematology,* 9th ed, Chap. 81, Table 81–10.

Venous Thrombosis

- There may be an increased incidence of venous thromboembolic disease in persons with HIV infection.

- The increased risk of thrombosis appears independent of concurrent malignancy.
- HIV infection may be associated with the development of a hypercoagulable state.
- Abnormalities of coagulation proteins observed in HIV-infected patients include acquired deficiencies in protein S or protein C.

Thrombotic Thrombocytopenia Purpura

- Thrombotic thrombocytopenia purpura (TTP) is associated with advanced AIDS.
- Factors associated with occurrence of TTP include higher HIV viral loads, lower CD4+ counts, and increased incidence of AIDS diagnoses, as well as infections with *Mycobacterium avium* complex and hepatitis C.
- The incidence of TTP is decreasing in the HAART era.

HIV-ASSOCIATED MALIGNANCIES

- More than 40% of all HIV-infected patients eventually are diagnosed with cancer. See **Table 51–6** for the most common HIV-associated malignancies and related oncogenic viruses.
- In the HAART era, malignancies account for 20% of deaths in persons with HIV-infection.
- Spectrum of neoplastic disease appears to be wider than initially thought.
- Three cancers currently considered AIDS-defining in HIV-infected persons are:
  — Kaposi sarcoma: associated with the epidemic from the onset in 1981
  — Intermediate- or high-grade B-cell lymphoma: added to the case definition for AIDS in 1985
  — Uterine cervical carcinoma: became an AIDS-defining illness in 1993
- There is increased risk of Hodgkin lymphoma among patients infected with HIV.

TABLE 51–6 AIDS-DEFINING MALIGNANCIES AND ONCOGENIC VIRUSES

AIDS-Defining Malignancy	Oncogenic Virus
Kaposi sarcoma	HHV-8
Aggressive non-Hodgkin lymphoma	EBV, HHV-8
Primary central nervous system lymphoma	EBV
Invasive cervical cancer	HPV

AIDS, acquired immunodeficiency syndrome; EBV, Epstein-Barr virus; HHV-8, human herpes virus 8; HPV, human papilloma virus.
Source: *Williams Hematology,* 9th ed, Chap. 81, Table 81-6.

AIDS-Related Lymphoma

Epidemiology

- Patients with AIDS have a risk of developing lymphoma that is nearly 100 times greater than that of the general population.
- The incidence of lymphoma increases with time after infection and may approach 20% for patients with prolonged, far-advanced immunodeficiency.
- In the United States, the relative risk of developing lymphoma within 3 years of an AIDS

diagnosis was increased by 165-fold compared to people without AIDS.

- Lymphomas have increased since the widespread use of HAART.
  — In contrast, HAART has led to a major and dramatic decline in the incidence of Kaposi sarcoma.
  — In both the pre-HAART and HAART periods, patients with lower CD4+ cell counts were more likely to develop lymphoma.
  — However, patients infected with HIV who maintain high CD4+ counts and good immune function with effective HAART therapy may have a reduced risk for developing lymphoma compared with HIV-infected patients with low CD4+ counts.

Pathology

- More than 80% of lymphomas associated with AIDS are intermediate- or high-grade B-cell tumors, including immunoblastic or large B-cell types and small noncleaved or Burkitt lymphomas (**Table 51–7**).
- The two most common histologic subtypes of lymphoma in HIV-infected patients are Burkitt lymphoma (see Chap. 64) and diffuse large B-cell lymphoma (see Chap. 60).

TABLE 51–7 CATEGORIES OF HIV-ASSOCIATED LYMPHOMAS: WORLD HEALTH ORGANIZATION CLASSIFICATION

Lymphomas also occurring in immunocompetent patients
Burkitt lymphoma
Classic
With plasmacytoid differentiation
Atypical
Diffuse large B-cell lymphoma
Centroblastic
Immunoblastic
Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) lymphoma (rare)
Peripheral T-cell lymphoma (rare)
Classic Hodgkin lymphoma
Lymphomas occurring more specifically in patients who are HIV positive
Primary effusion lymphoma
Plasmablastic lymphoma of the oral cavity
Lymphomas occurring in other immunodeficiency states
Polymorphic B-cell lymphoma

HIV, human immunodeficiency virus.
Source: *Williams Hematology,* 8th ed, Chap. 83, Table 83–3.

Burkitt Lymphoma

- In some cases, the cells have a plasmacytoid appearance, characterized by medium-size cells with abundant cytoplasm and eccentric nuclei.
- This type of Burkitt lymphoma is termed *Burkitt lymphoma with plasmacytoid differentiation* in the World Health Organization (WHO) classification, an entity unique to patients with HIV.

Diffuse Large B-cell Lymphoma

- In the WHO classification, AIDS-related diffuse large B-cell lymphomas are divided into

centroblastic and immunoblastic subtypes.
- Compared to the centroblastic subtype, the immunoblastic subtype more frequently involves extranodal sites, particularly the central nervous system (CNS), and is more commonly associated with Epstein-Barr virus infection.

Primary Effusion Lymphoma
- Primary effusion lymphoma and plasmablastic lymphoma of the oral cavity occur principally in patients with HIV infection.
- Primary effusion lymphoma is uncommon, representing only a small fraction of all AIDS-related lymphomas, is caused by HHV-8.

T-Cell Lymphoma
- Patients with AIDS are at an increased risk for developing T-cell lymphomas.
- The prevalence of T-cell lymphomas among patients with AIDS-related lymphoma is approximately 3%.

Clinical Features
- B symptoms, such as fever, night sweats, and weight loss, are present at diagnosis in 80% to 90% of patients with AIDS-related lymphoma, and 61% to 90% have far-advanced disease presenting in extranodal sites.
- Virtually any anatomic site may be involved.
  — The more common sites of initial extranodal disease include the CNS (17%–42%), gastrointestinal tract (4%–28%), marrow (21%–33%), and liver (9%–26%).
  — Staging evaluation should include computed tomography (CT) scanning of the chest, abdomen, and pelvis; a gallium-67 scan or positron emission tomography (PET) scan; marrow aspirate and biopsy; and other studies as clinically indicated.
  — Lumbar puncture should routinely be performed because approximately 20% of patients have leptomeningeal lymphoma, even in the absence of specific symptoms or signs.
  — Intrathecal methotrexate or cytosine arabinoside is often given to prevent isolated CNS relapse.

Primary CNS Lymphoma
- Approximately 75% of patients with primary CNS lymphoma have far-advanced HIV disease, with median CD4+ cell counts less than 50/μL, and a prior history of AIDS.
- Initial symptoms and signs may be variable, with seizures, headache, and/or focal neurologic dysfunction noted in most patients.
- Radiographic scanning reveals relatively large mass lesions (2–4 cm), which tend to be few in number (one to three lesions). Ring enhancement may be seen.
- There is no specific radiographic picture.
- PET scanning may be useful in differentiating cerebral lymphoma, which has a glucose uptake above that of the surrounding cortex. This is in contrast to toxoplasmosis, which has a glucose uptake below that of the cerebral cortex.

- In addition, thallium-201 single-photon emission computed tomography scanning may be useful, with a median T1 uptake index greater than 1.5 and a lesion size greater than 2.5 cm serving as independent predictors of primary CNS lymphoma.
- Pathologically, almost all such lymphomas are of diffuse large B-cell or immunoblastic subtypes and are uniformly associated with Epstein-Barr virus infection within malignant cells.
- Detection of Epstein-Barr virus DNA (Epstein-Barr nuclear antigen) in cerebrospinal fluid by PCR may be used as a diagnostic criterion.
- Use of cranial radiation is associated with a complete remission rate of only 50% and median survival of only 2 or 3 months.
- Use of HAART is associated with significantly prolonged survival.

T-Cell Lymphomas

- Systemic B symptoms, consisting of fever, drenching night sweats, and/or unexplained weight loss, are extremely common in patients with T-cell lymphomas.
- T-cell lymphomas also present with advanced lymphomatous disease, with stage IV disease confirmed in up to 90%.

Primary Effusion Lymphoma

- Outcome with polychemotherapy has generally been poor, with median survival of approximately 2 months.
- Studies reported complete remissions in patients treated with HAART alone.
- Palliative measures include draining effusions and therapeutic radiation to affected areas.

Prognostic Markers in AIDS-Related Lymphoma

- The age-adjusted international prognostic index (IPI) established for immunocompetent patients with intermediate-grade lymphoma is also predictive of outcome in AIDS patients with lymphoma (see Chap. 61).
- High risk group and low CD4+ cell count are the two predictors of poor survival.
- Histology of Burkitt lymphoma was an independent poor prognostic factor for survival.
- Low IPI and postgerminal center differentiation were identified as independent prognostic factors for relatively long disease-free survival.
- Patients with systemic lymphoma with leptomeningeal involvement have decreased survival.

Treatment

- AIDS Clinical Trials Group in the United States compared standard-dose m-BACOD and GM-CSF support with reduced-dose m-BACOD without granulocyte-monocyte colony-stimulating factor in 198 HIV infected patients with aggressive lymphomas.
  - — No significant differences were found in either response rate (standard dose 52% vs reduced dose 41%) or median survival (standard dose 6.8 months vs reduced dose 7.7 months).
  - — However, reduced dose m-BACOD was associated with a statistically significant lower

toxicity.

- The EPOCH regimen consists of a 96-hour continuous infusion of etoposide, prednisone, vincristine, and doxorubicin, and a bolus of cyclophosphamide, which was dose adjusted based on the patient's CD4+ cell count and neutrophil count at the nadir.
  - The overall complete remission rate was 74%. Among patients with CD4+ cell counts greater than 100/µL, the complete remission rate was 87%, and the overall survival was 87% at 56 months.
  - A particular survival advantage in patients with CD4+ cell counts of less than 100/µL was reported by adding rituximab to EPOCH.
  - R-EPOCH (rituximab on days 1 and 5) was given to 21 subjects with AIDS-related lymphoma. Persons with CD4+ cell counts greater than 100/µL fared similarly after EPOCH with or without rituximab, whereas persons with CD4 cell counts less than 100/µL had survival of 57% with R-EPOCH versus 16% with EPOCH alone.
  - Rituximab increased the risk of severe and life-threatening infection when used in combination with chemotherapy in patients with AIDS.
- Concurrent use of HAART during administration of chemotherapy is generally tolerated.
  - Use of HAART is associated with improved survival in patients with AIDS-related lymphoma.
  - Delaying HAART until completion of chemotherapy is reasonable for patients with CD4+ counts greater than 100/µL but does not appear to be necessary.
  - Including HAART therapy with chemotherapy in patients with CD4+ counts less than 100/µL cells clearly seems important given the poor survival rates in this group when HAART is not used.
- With the advent of HAART and improvement in supportive care, HIV-infected patients with relapsed or refractory lymphoma now can be effectively retreated with high-dose chemotherapy and peripheral stem cell transplantation.

Hodgkin Lymphoma in the Setting of HIV Infection

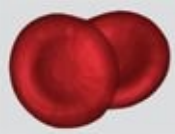
- HIV-related Hodgkin lymphoma seems to be associated with more profound immunodeficiency.
- Paradoxically, the highest risk of HIV-related Hodgkin lymphoma is found in persons with CD4+ counts between 225 and 250 cells/µL, which are above the level required to establish an immunologic AIDS diagnosis.
- The risks decline with CD4+ cell counts both above and below that range, with the lowest risks seen with counts less than 75 CD4+ cells/µL.
- HIV-related Hodgkin lymphoma is characterized by the preponderance of more aggressive histologic subtypes, with mixed cellularity Hodgkin lymphoma and lymphocyte depletion Hodgkin lymphoma diagnosed in 41% to 100% of patients.
- Another distinguishing feature of HIV-related Hodgkin lymphoma is its close association with Epstein-Barr virus.
- Systemic B symptoms such as fever, drenching night sweats, and/or weight loss occur in 70% to 100% of patients with HIV-related Hodgkin lymphoma compared with 30% to 60% of patients with de novo Hodgkin lymphoma.
- Marrow involvement is present in 50% of patients with underlying HIV infection, often

presenting with pancytopenia and systemic B symptoms.

- Staging evaluation should include a thorough history; physical examination; standard laboratory tests; CT scans of the chest, abdomen, and pelvis; gallium or PET scans; and bilateral marrow biopsies.
- With the availability of HAART, better treatment outcomes with combination chemotherapy have been reported.
- Although improved outcomes have been reported in the HAART era, results still are inferior compared with those in HIV-negative patients with Hodgkin lymphoma, even among those with stage IV disease.

Multicentric Castleman Disease in the Setting of HIV Infection

- Multicentric Castleman disease (MCD) is a diffuse lymphoproliferative disorder.
- MCD is characterized histologically by angiofollicular hyperplasia and plasma cell infiltration.
- MCD manifests itself as a systemic syndrome with elevated interleukin-6 and C-reactive protein. The syndrome may flare for several days to weeks, resolving spontaneously at times.
- Clinical features include lymphadenopathy, splenomegaly, fevers, weight loss, hypotension, pancytopenia, hypoalbuminemia, and oligoclonal or monoclonal gammopathy.
- In the setting of HIV, persons with MCD are at increased risk for both Kaposi sarcoma and lymphoma.
- Neither HIV viral load nor CD4+ cell count has been predictive of the risk of developing MCD, MCD flares, or MCD-related lymphoma.
- MCD is universally associated with HHV-8.
- Improvements and exacerbations after initiating HAART have been described.
- MCD in patients infected with HIV is often progressive and potentially fatal.



For a more detailed discussion, see Virginia C. Broudy and Robert D. Harrington: Hematologic Manifestations of Acquired Immunodeficiency Syndrome, Chap. 81 in *Williams Hematology*, 9th ed.

CHAPTER 52

The Mononucleosis Syndromes

DEFINITION

- Infectious mononucleosis is defined as any blood lymphocytosis induced in response to an infectious agent.
- Usually greater than 50% of the circulating white cells are lymphocytes, more than 10% of which have the morphology of reactive lymphocytes (**Figure 52–1**).
- **Table 52–1** lists the etiologic agents that produce mononucleosis.
- Pharyngeal form:
  — A sore throat is preceded by 1 to 2 weeks of lethargy.
  — Epstein-Barr virus (EBV) generally is the cause.
- Glandular form without pharyngitis:
  — Lymph node enlargement occurs.
  — The usual cause is agents other than EBV (eg, *Toxoplasma gondii*).
- Typhoidal form:
  — Lethargy with fever or diarrhea without pharyngitis, usually as a consequence of cytomegalovirus (CMV), is characteristic.

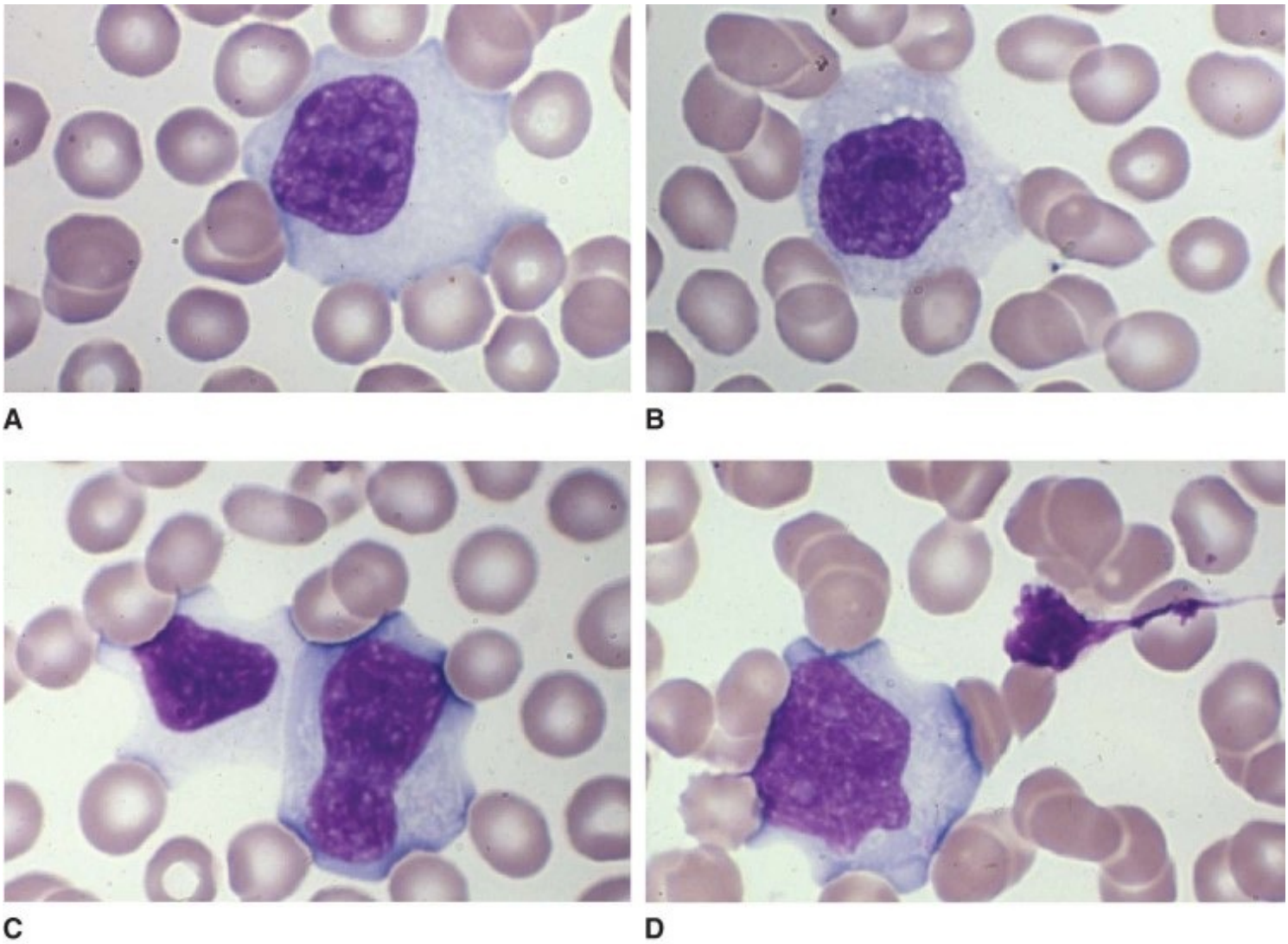


FIGURE 52–1 **A–D.** Blood films from patients with Epstein-Barr virus–induced mononucleosis. These reactive lymphocytes exhibit the characteristic changes seen in patients with infectious mononucleosis: large lymphocytes with abundant cytoplasm. The cytoplasmic margin often spreads around (is indented by) neighboring red cells and the margin may take on a densely basophilic coloration. This type of reactive T lymphocyte may be seen a variety of diseases and is not specific changes but are characteristic. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

TABLE 52–1 **ETIOLOGIC AGENTS ASSOCIATED WITH MONONUCLEOSIS SYNDROME**

Epstein-Barr virus	Hepatitis A
Cytomegalovirus	Adenovirus
Human immunodeficiency virus	*Toxoplasma gondii*
Human herpes virus-6	*Bartonella henselae*
Metapneumovirus	*Brucella abortus*
Rubella	

Source: *Williams Hematology,* 9th ed, Chap. 82, Table 82–1.

ETIOLOGY AND PATHOGENESIS

- The caused is two members of the herpes virus family: EBV or CMV.
- After the early phase of fever, which lasts for 3 to 7 days, laboratory abnormalities include a

blood lymphocyte proportion greater than 50%, often with greater than 10% reactive lymphocytes.

- Table 52–2 lists other complications of EBV and CMV mononucleosis.

TABLE 52–2 COMPLICATIONS IN PATIENTS WITH EBV OR CMV MONONUCLEOSIS

	EBV	CMV
Hemolytic anemia	++	+
Thrombocytopenia	+	+
Aplastic anemia	+	–
Splenic rupture	+	–
Jaundice (> age 25 years)	++	++
Guillain-Barré*	+	++
Encephalitis*	++	+/–
Pneumonitis*	+/–	+
Myocarditis*	+	–
B-cell lymphoma	+	–
Agammaglobulinemia	+	–

*Can occur without mononucleosis syndrome. ++, common; +, infrequent; +/–, uncommon; –, not observed.
Source: *Williams Hematology,* 9th ed, Chap. 82, Table 82–2.

FEATURES OF MONONUCLEOSIS CAUSED BY EACH ETIOLOGIC AGENT

- Table 52–3 list the signs and symptoms associated with EBV and CMV mononucleosis.
- Target cell for EBV mononucleosis is the B lymphocyte.
- Target cell for CMV mononucleosis is the macrophage.
- The “mononucleosis” for both is the increase in reactive blood T lymphocytes.
- Hepatosplenomegaly common for both EBV and CMV mononucleosis.
- Incubation period for EBV or CMV is 30 to 50 days.

TABLE 52–3 SIGNS AND SYMPTOMS OF EBV AND CMV: EFFECT OF AGE

	Percent of Subjects		
Signs and Symptoms	**EBV (Age 14–35 Years)**	**EBV (Age 40–72 Years)**	**CMV (Age 30–70 Years)**
Fever	95	94	85
Pharyngitis	95	46	15
Lymphadenopathy	98	49	24
Hepatomegaly	23	42	N/A
Splenomegaly	65	33	3
Jaundice	8	27	24

CMV, cytomegalovirus; EBV, Epstein-Barr virus.
Source: *Williams Hematology,* 9th ed, Chap. 82, Table 82–3.

EBV MONONUCLEOSIS

Virology and Pathogenesis

- DNA virus of the gammaherpesvirinae subfamily.
- The virus infects 90% of the world population.
- Peak incidence occurs in the age group from 12 to 25 years and during the summer months.
- B lymphocytes are the initial target of EBV during primary infection.
- Surface receptor for EBV is CD21 on B cells.
- Initial infection causes polyclonal or oligoclonal B-cell proliferation.
- Neoantigen(s) on EBV-infected B cells induces a cytotoxic T-cell response.
- Most circulating lymphocytes are reactive T cells.
- Cytotoxic T cells destroy most EBV-infected B cells leading to disease resolution.
- Following infection, the virus persists throughout life in a latent form.

Epidemiology

- Transmission requires close mucocutaneous contact.
- In the developing world and in the lowest socioeconomic strata of the developed world, nearly everyone is subclinically infected by age 5 years and mononucleosis is rarely clinically apparent.
- In the upper socioeconomic strata of the developed world, persons avoid infection in infancy; instead, they become exposed to the virus between the ages of 12 and 25 years by contact with a latently infected asymptomatic individual.
- Individuals who are raised in more protected environments or in single-child families may reach an age of 30 years or older before they are infected.

Clinical Manifestations

- Clinical features vary by age:
  - — When young children acquire infection with EBV, they develop a typical childhood illness of respiratory tract infection (43%), otitis media (29%), pharyngitis (21%), gastroenteritis (7%), or typical mononucleosis (< 10%).
  - — In older children and young adults, age group 12 to 25 years, the earliest manifestations of disease—fever and lassitude—develop 30 to 45 days after patients become infected. Initial symptoms of pharyngitis, tonsillar enlargement, sometimes massive, and fever result from infection and proliferation of the B lymphocytes that are found in the pharyngeal of the Waldeyer ring of the lymph nodes.
- Liver function abnormalities, usually cholestatic, are frequently present.
- Maculopapular rash with EBV mononucleosis can be worsened by administration of ampicillin or amoxicillin.
- Group A streptococcus infection may occur coincidentally but does not affect the disease and its usual course.
  - — Penicillin or erythromycin is indicated if group A streptococcus isolated from throat cultures of symptomatic patients.
- Complications caused by immune dysregulation or lymphocyte proliferation:

— Immune thrombocytopenic purpura (ITP) or autoimmune hemolytic anemia
— Splenic rupture
— Acute airway obstruction resulting from exaggerated pharyngeal lymphadenopathy
— B-cell lymphoproliferative disorder/lymphoma in immunosuppressed patients

- Disease abates with the occurrence of a T-cell-mediated counter-response to the virus-induced polyclonal B-cell proliferation and clinical improvement occurs within 24 to 48 hours in most cases.

Laboratory Findings

Table 52–4 lists the laboratory abnormalities for EBV and CMV mononucleosis.

TABLE 52–4 LABORATORY ABNORMALITIES IN MONONUCLEOSIS SYNDROME

	Frequency	
	EBV	**CMV**
Heterophile antibody	+++	–
Lymphocytosis	+++	++
Reactive lymphocytes	+++	++
Abnormal liver function	++	++
Antinuclear factor	+	+
Cold agglutinins	+	+
Cryoglobulins	+	+
Decreased platelets	++	+

+++, Characteristic; ++, common; +, occurs; CMV, cytomegalovirus; EBV, Epstein-Barr virus.
Source: *Williams Hematology,* 9th ed, Chap. 82, Table 82–5.

Antibody Responses

- Heterophile antibody is positive only with EBV.
- Autoantibodies:
  — Cold agglutinins occur frequently with EBV infection.
- Antibody tests for EBV:
  — Antibodies to EBV do not react with CMV or with the heterophile antigen.
  — IgM and IgG antivirus capsid antigen (VCA) appear during acute illness (IgM persists for months, IgG for life).
  — Early antigen (EA) specific antibodies appear slightly later than IgG anti-VCA and persist for years.
  — Antibodies to Epstein-Barr nuclear antigen (EBNA) do not develop until after the acute illness resolves and persist for life.
  — A presumptive diagnosis of EBV infectious mononucleosis may be made if the patient has antibodies specific for VCA, but not for EBNA.

Reactive Lymphocytes

- Expansion of cytotoxic T lymphocytes produces lymphocytosis. Reactive lymphocytes are

larger than lymphocytes normally found in the blood (see **Figure 52–1**).
- Reactive lymphocytes are a hematologic hallmark of infectious mononucleosis, but they are not always found and are not pathognomonic.

Other Blood Test Abnormalities

- Liver function abnormalities are common, predominantly elevated serum alkaline phosphatase and γ-aminotransferase activity with no or only slight elevation of bilirubin in most patients.

Course and Prognosis

Complications of EBV Mononucleosis

- Hematologic:
  — These occur infrequently but include severe immune thrombocytopenia with petechiae, immune hemolytic anemia, immune-mediated agranulocytosis, and aplastic anemia.
- Neurologic:
  — Patients may occasionally develop encephalitis, acute disseminated encephalomyelitis (*Alice in Wonderland* syndrome), acute cerebellar ataxia, viral meningitis, Guillain-Barré syndrome, transverse myelitis, and cranial nerve palsies.
  — Other complications that may be associated are chronic fatigue, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, and chronic progressive EBV infection, T or NK lymphoproliferation, lymphoma, and hemophagocytic syndrome.

Other EBV-Associated Disease Processes

Neoplastic Potential of the Virus

- The virus has been associated Burkitt lymphoma and other tumors (see **Table 52–5**).
- It is detectable in neoplastic B cells (Reed-Sternberg cells) of approximately 35% of patients with Hodgkin lymphoma, and its etiologic role is uncertain.
- Because of the severe consequences of EBV infection, several approaches to preventing or treating these disorders are under way, including:
  — Development of an EBV vaccine
  — Adoptive transfer of activated cytotoxic T cell
  — Development of peptides that inhibit viral replication

TABLE 52–5 SPECIAL PROBLEMS WITH EBV OR CMV

EBV	CMV
Rare congenital infection	Congenital infection
Chronic progressive mononucleosis	Post-transplant primary infection
Hemophagocytic syndrome	Graft-versus-host disease association
X-linked B-cell lymphoma	Transfusion-related infection
Posttransplant lymphoproliferative disease	*Aspergillus* and/or *Pneumocystis* infection
T or natural killer cell lymphoproliferative disease	
African Burkitt lymphoma	

Approximately 20% of Burkitt lymphoma in the United States
Approximately 35% of Hodgkin lymphoma
Nasopharyngeal carcinoma
Approximately 5% of gastric carcinoma
Leiomyoma and leiomyosarcoma in HIV or immunosuppressed patients
Oral hairy leukoplakia

CMV, cytomegalovirus; EBV, Epstein-Barr virus; HIV, human immunodeficiency virus.
Source: *Williams Hematology*, 9th ed, Chap. 82, Table 82–4.

CMV MONONUCLEOSIS

- CMV is the second most common cause of infectious mononucleosis.

Epidemiology

- Teenage mothers carrying CMV in their cervix transmit it to their newborn child, and transmission also occurs through breast milk.
- Transmission from contact with infected young children also plays a role.
- Sexual transmission is also a factor.

Clinical Manifestations

- See **Table 52–3** for the major clinical findings and **Table 52–2** for the principal complications of CMV mononucleosis.
- The basic clinical disease is fever, often as high as 40°C (104°F), with a palpable spleen and laboratory abnormalities.
- CMV mononucleosis commonly occurs in older individuals, often those older than 50 years.
- Reactive lymphocytosis is a result of T cells reacting against CMV-infected monocytes/macrophages (see **Table 52–4**).

Laboratory Findings

- See **Table 52–4**.
- Polyclonal antibody and heterophile antibody responses do not occur, but specific anti-CMV antibodies do develop.
- Because the incubation period ranges between 30 and 40 days, IgM and IgG antibodies to CMV usually are positive at presentation.
- Tests for CMV:
  — Primary infection diagnosed by fourfold rise in anti-CMV antibody titer
  — Assay for CMV antigenemia more sensitive than anti-CMV antibody titer
  — Polymerase chain reaction (PCR) for detection of CMV DNA, which is most sensitive

Complications

- Hemolytic anemia and thrombocytopenia occur in primary CMV infection and are other factors that may lead the clinician initially to consider a diagnosis of lymphoma.

- Various neurologic complications can occur, but Guillain-Barré syndrome is the most frequent and is usually associated with CMV infection.

PRIMARY HIV INFECTION

- Mononucleosis can occur soon after primary infection (see Chap. 51).
- Mononucleosis symptoms are self-limited but may last for several weeks.
- Leukopenia, thrombocytopenia, a relative increase in band neutrophils, and a small proportion of reactive lymphocytes usually can be identified on the blood.
- Lymphocytosis is uncommon.

OTHER AGENTS LINKED TO MONONUCLEOSIS SYNDROME

- Herpes virus-6
- Varicella zoster
- Hepatitis A or B
- Rubella
- Adenovirus
- *T gondii:*
  - — This species is the only nonviral agent commonly identified as causing a mononucleosis syndrome.
  - — Infection occurs secondary to ingestion of cysts in raw meat or of oocysts in cat feces.
  - — There is no documented person-to-person transmission.
  - — Asymptomatic or isolated lymphadenopathy without fever usually occurs.
  - — Patients do not commonly have pharyngitis.

DIFFERENTIAL DIAGNOSIS

- Acute pharyngitis can be caused by infection with β-hemolytic streptococcus, adenovirus, or *Arcanobacterium haemolyticum.*
- Fever, lymphocytosis, and splenomegaly may raise consideration of lymphoma.
- CMV infection can be associated with presence of antinuclear antibodies similar to those of patients with new-onset systemic lupus erythematosus.
- Mononucleosis syndrome of toxoplasmosis can be distinguished from that caused by other infections by presence of high-titer antitoxoplasma antibodies.
- Patients with mononucleosis syndrome secondary to hepatitis virus infection generally have abnormal liver function tests.

TREATMENT AND COURSE

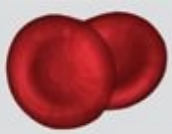
- Disease is usually self-limited.
- Acetaminophen is useful for fever and pharyngeal pain and gargling with saline is useful for

pharyngitis.

- Give prednisone 40 to 60 mg/day for 7 to 10 days, then taper dose over 1 week for severe or life-threatening complications, such as:
  — Imminent upper airway obstruction
  — Immune thrombocytopenia purpura
  — Immune hemolytic anemia
  — Central nervous system involvement
- Acyclovir generally is ineffective in the treatment of infectious mononucleosis.
- Ganciclovir may be beneficial for immunocompromised patients or in patients with severe, complicated primary EBV mononucleosis.
- Ganciclovir (5 mg/kg day for 14 days) is effective against CMV but recommended only for patients with severe disease and/or who are immunocompromised.
- Antiretroviral therapy for primary HIV-1 infection can clear viremia and restore CD4 lymphocytes (see Chap. 51).

Mononucleosis in Pregnancy

- Abortion may be considered for any pregnant woman who develops infectious mononucleosis as a result of primary infection with EBV, CMV, or toxoplasmosis, especially during the first trimester.
- EBV mononucleosis during gestation can produce severe congenital anomalies, including microcephaly, hepatosplenomegaly, cataracts, mental retardation, or death.
- About half of the infants born to mothers who develop primary CMV infection during pregnancy will have congenital infection. Of these, about one-fourth will be symptomatic and/or have congenital anomalies.
- Primary toxoplasmosis infection in first trimester also can result in congenital abnormalities.
- Mothers with antitoxoplasmosis antibodies before pregnancy do not transmit the organism to the developing infant.
- HIV-1 can be transmitted to the infant during primary infection and should be treated with zidovudine alone or in combination with elective caesarean section to reduce the rate of maternal–infant HIV-1 transmission (see Chap. 51).



For a more detailed discussion, see Robert F. Betts: Mononucleosis Syndromes, Chap. 82, in *Williams Hematology*, 9th ed.

PART VIII

THE CLONAL LYMPHOID AND PLASMA CELL DISEASES

CHAPTER 53

Classification and Clinical Manifestations of the Malignant Lymphoid Disorders

CLASSIFICATION

- Lymphocyte malignancies comprise a wide spectrum of different morphologic and clinical syndromes.
- The International Lymphoma Study Group proposed a new classification termed **R**evised **E**uropean–**A**merican **L**ymphoma classification, or REAL classification, which was modified in 2001 and updated in 2008 by the World Health Organization (WHO) (see **Table 53–1**).
- The REAL/WHO classification makes use of the pathologic, immunophenotypic, genetic, and clinical features to define separate disease entities (**Table 53–1**).
- Lymphocytic neoplasms are divided into those derived from B cells, T cells, or natural killer (NK) cells.
- The immunophenotype and the characteristic cytogenetic and genic abnormalities are also included in **Table 53–1**.
- The most common indolent and aggressive lymphocytic neoplasms, based on their usual clinical behavior, are listed in **Tables 53–2** and **53–3**.

TABLE 53–1 CLASSIFICATION OF LYMPHOMA AND LYMPHOID LEUKEMIA BY THE WORLD HEALTH ORGANIZATION

Neoplasm	Morphology	Phenotype*	Genotype†
B-Cell Neoplasms			
Immature B-Cell Neoplasms			
Lymphoblastic leukemia/lymphoma not otherwise specified (NOS) (Chap. 54)	Medium to large cells with finely stippled chromatin and scant cytoplasm	TdT+, sIg-, CD10+, CD13+/-, CD19+, CD20-, CD22+, CD24+, CD34+/-, CD33+/-, CD45+/-, CD79a+, PAX5+	Clonal DJ rearrangement of *IGH* gene T(17;19), *E2A-HLF, AML1* iAMP21 associated with poor prognosis
Lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities (Chap. 54)	See above	See above. B-ALL with t(9;22) with CD25 and more frequent myeloid antigens CD13, CD33	See individual genetic features in B-ALL subtypes below
B-ALL with t(v;11q23); *MLL* rearranged	See above	CD19+, CD10-, CD24-, CD15+	Multiple MLL (11q23) fusion partners including *AF4* (4q21), *AF9* (9p22), and *ENL* (19p13). B-ALL with MLL translocations over express *FLT-3*. Poor prognosis
B-ALL with t(12;21) (p13;q22);	See above	CD19+, CD10+, CD34+.	t(12;21)(p13;q22) *ETV6-RUNX*

TEL-AML1 (*ETV6-RUNX1*)		Characteristically negative for CD9, CD20, and CD66c	translocation
B-ALL with hyperdiploidy	See above	CD19+, CD10+, CD45-, CD34+	Numerical increase in chromosomes without structural abnormalities. Most frequent chromosomes +21, X, 14 and 4. +1,2, 3 rarely seen. Favorable prognosis
B-ALL with hypodiploidy	See above	See above	Loss of at least one or more chromosomes (range from 45 chromosomes to near haploid). Rare chromosome abnormalities. Poor prognosis
B-ALL with t(5;14) (q31;q32); *IL3-IGH*	See above (with reactive eosinophilia)	See above. Even rare blasts with B-ALL immunophenotype with eosinophilia strongly suggestive of this subtype of B-ALL	t(5;14)(q31;q32); *IL3-IGH* leading to overexpression of IL3. Unclear prognosis
B-ALL with t(1;19) (q23;p13.3); *E2A-PBX1*	See above	CD10+, CD19+, cytoplasmic μ heavy chain. CD9+, CD34-	t(1;19)(q23;p13.3); leads to overexpression of *E2A-PBX1* fusion gene product interfering with normal transcription factor activity of E2A and PBX1
Mature B-Cell Neoplasms			
Leukemias			
Chronic lymphocytic leukemia/small lymphocytic lymphoma (Chap. 55)	Small cells with round, dense nuclei	sIg+(dim), CD5+, CD10-, CD19+, CD20+(dim), CD22+(dim), CD23+, CD38+/-, CD45+, FMC-7-	IgR+, trisomy 12 (~30%), del at 13q14 (~50%), 11q22–23, 17p13, and *IGHV* mutated status associated with poor prognosis
Prolymphocytic leukemia (Chap. 55)	≥ 55% prolymphocytes	sIg+(bright), CD5+/-, CD10-, CD19+, CD22+, CD23+/-, CD45+, CD79a+, FMC7+	del13q.14(~30%); del17p (50%), IgR+
Hairy cell leukemia (Chap. 56)	Small cells with cytoplasmic projections	sIg+(bright), CD5-, CD10-, CD11c+(bright), CD19+, CD20+, CD25+, CD45+, CD103+, Annexin A+	BRAF mutations (~100%), IgR+
Lymphomas			
Lymphoplasmacytic lymphoma (Chap. 69)	Small cells with plasmacytoid differentiation	cIg+, CD5−, CD10−, CD19+, CD20+/− Plasma cell population: CD38+, CD138+, cIgM+	IgR, 6q- in 50% of marrow-based cases [the t(9;14) was proved to be wrong], +4 (20%)
Mantle cell lymphoma (Chap. 62)	Small to medium cells	sIgM+, sIgD+, CD5+, CD10-, CD19+, CD20+, CD23-, Cyclin D1+, FMC-7+, SOX11+ in nearly 100% of cases	IgR, t(11;14)(q13;q32) (~100% by FISH), involving *BCL1* and IgH. Highly proliferative variants often show TP53 mutation, deletion of *INK4a/ART* and *p18INK4c*
Follicular lymphoma (follicle	Small, medium, or large cells	sIg, CD5–, CD10+, CD19+,	IgR, t(14;18)(q32;q21) (~85%)

center lymphoma; Chap. 61)	with cleaved nuclei	CD20+(bright), CD23-/+, CD38+, CD45+	involving *BCL2* and IgH. Mutated 3q27 (5–15%, *BCL6*)
Nodal marginal zone B-cell lymphoma (Chap. 63)	Small or large monocytoid cells	sIgM+, sIgD–, cIg+ (~50%), CD5-, CD10-, CD11c+/-, CD19+, CD20+, CD23-, CD43+/-	IgR, commonly with trisomies 3, 7, and 18
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT) type (Chap. 63)	See above	See above	t(11;18)(q21;q21) involving *API2*, *MLT1*, or t(1;14)(p22;q32) involving *BCL10*
Splenic B-cell marginal zone lymphoma	Small round lymphocytes replaces reactive germinal centers and/or villous lymphocytes in blood	sIgM+, sIgD-, CD5+/-, CD19+, CD20+, CD23-, CD103-	IgR, allelic loss of chromosome 7q31– 32 (40%)
B-Cell Neoplasms			
Splenic B-cell lymphoma, unclassifiable			
Splenic diffuse red pulp small B-cell lymphoma	Blood: villous lymphocytes similar to SMZL. Marrow: intrasinusoidal infiltration. Spleen: monomorphous small to medium lymphocytes with round nuclei, vesicular chromatin, occasional small nucleoli	CD20+, DBA.44+, IgG+/IgD-, CD25-, CD5-, CD103-, CD123-	T(9;14)(p13;q32) involving *PAX5* and *IGH*
Hairy cell leukemia variant	Hybrid features of prolymphocytic leukemia and classic hairy cell leukemia	sIg+(bright), CD5-, CD10-, CD11c+(bright), CD19+, CD20+, CD25-, CD45+, CD103+, FMC7+, CD123-, Annexin A1-, TRAP–	BRAF mutation negative
Diffuse large B-cell lymphoma (DLBCL, Chap. 60)			
DLBCL NOS			
Common morphologic variants:			
Centroblastic	Medium to large lymphoid cells with vesicular nuclei containing fine chromatin. Multiple nucleoli	sIgM+, sIgD+/-, CD5-, CD10-/+, CD19+, CD20+, CD22+, CD79a+, CD45+, PAX5+	IgR, 3q27 abnormalities and/or t(3;14) (q27;q32) involving *BCL6* (~30%) or t(14;18) (q32;q21) (~25%) involving *BCL2*
Immunoblastic	> 90% of cells are immunoblasts with central nucleolus	See above. May express CD30+	See above
Anaplastic	Very large round, oval, or polygonal cells with bizarre pleomorphic nuclei resembling R-S cells	See above. Often CD30+	See above
Molecular subgroups			
Germinal center B-cell–like (GCB)	See above	See above	See above
Activated B-cell–like (ABC)	See above. Often with more immunoblastic morphology	See above	T(14;18) (35%), 12q12 (20%), IG mutation, BCL2 rearrangement (20%–25%),

			Rel amplification (15%). Amplification of microRNA-17-92 cluster
Immunohistochemical subgroups		Gain of 3q (26%), 9p (6%), 12q12 (5%), NF-κB activation	
CD5-positive DLBCL	See above	See above. CD5+	t(11;14) and t(14;18) negative. +3 and gain on chromosome 16/16p and 18/18q common. Deletion p16/INK4a
Nongerminal center B-cell–like (non-GCB)	See above	See above	See above. Uniform FOXP1 expression with IRF4/MUM1 and BCL6 expression
Primary mediastinal (thymic) large B-cell lymphoma (Chap. 60)	Variable from case to case. Medium to large cells often with pleomorphic nuclei (R-S–like cells)	sIg-, CD5-, CD10-/+, CD15-, CD19+, CD20+, CD22+, CD23+, CD30+ (80%), CD45+, CD79a+, IRF4/MUM1 (75%). Variable BCL2 (50%–80%) and BCL6 (45%–100%) expression	IgR+, Gain of 9q24 (75%), gain 2p15 (50%) Amplification of *REL, BCL11A, JAK2, PDL1, PDL2*. Transcriptome similar to CHL
Intravascular large B-cell lymphoma	Neoplastic cells infiltrated within small to intermediate vessels of all organs	CD19+, CD20+, CD5 (38%), CD10 (13%). Lack of CD29 (β1 integrin) and CD54 (ICAM1) may account for intravascular growth pattern	IgR+, otherwise poorly characterized
ALK-positive large B-cell lymphoma	Sinusoidal growth pattern, monomorphic large immunoblast-like cells	Strongly positive for ALK, CD138+, VS38+, cytoplasmic IgA or IgG	IgR+, t(2;17) *ALK/CLTC*
Plasmablastic lymphoma	Diffuse proliferation of immunoblasts with plasmacytic differentiation, frequent mitotic figures, monomorphic morphology common in HIV+ patients. Frequently extranodal, EBV+	CD138+, CD38+, VS38C, IRF4/MUM1+, high Ki67, CD79a+ CD30+ in most cases. Negative for CD45, CD20, PAX5. Cytoplasmic Ig (50–70%). CD56 negative (if positive, suspect plasma cell myeloma)	IgR+, frequently Epstein-Barr virus-encoded RNA (EBER)+ (60%–70%) but most cases negative for LMP1. HHV8+ status consistent with large B-cell lymphoma from MCD (below)
Large B-cell lymphoma arising from multicentric, HHV8+ Castleman disease (MCD)	HHV8 MCD: B cell follicles with involution and hyalinization of germinal centers with prominent mantle zones. Large plasmablastic cells within mantle zone HHV8 plasmablastic lymphoma. Confluent sheets of HHV8+ LANA1+ cells effacing lymph node architecture. Extranodal involvement common	HHV8+, LANA1+, viral IL6+, cytoplasmic IgM, CD20+/-, Negative for CD79a, CD138, and EBV (EBER)	Polyclonal IgM. IgVH unmutated. IL6R pathway activation. Cytogenetics poorly characterized
B-Cell Neoplasms			
Primary effusion lymphoma	Range of infiltrating cells with highly abnormal morphology including immunoblastic, plasmablastic, anaplastic.	CD45+, Lack expression of CD19, CD20, CD79a, sIg	IgR+ and hypermutated. No recurrent chromosomal anomalies

	Large nuclei with prominent nucleoli		
Burkitt lymphoma (Chap. 64)	Medium cells arranged in diffuse, monotonous pattern. Basophilic cytoplasm, high proliferative index with frequent mitotic figures. "Starry sky" pattern present	Positive for CD19, CD20, CD10, BCL6, CD38, CD77, and CD43. Negative for BCL2 and TdT. Ki67+ in nearly 100% of tumor cells	t(8;14)(q24;q32), t(2;8)(q11;q24), or t(8;22)(q24;q11), involving Ig loci and *C-MYC*at 8q24
B-cell lymphoma unclassifiable, features intermediate between DLBCL and Burkitt lymphoma (BL) (Chap. 64)	Medium, round cells with abundant cytoplasm. More variation in nuclear size and contour compared to BL. Commonly > 90% Ki67+. Unlike BL, can show strong BCL2 expression	Same as above except sIg-, cIg+/-, and CD10-	Same as above except more typically expresses high levels of *BCL2* and ~30% have *BCL2* rearrangements (double-hit type)
B-cell lymphoma unclassifiable, features intermediate between DLBCL and classical Hodgkin lymphoma (HL)	Confluent, diffuse, sheet-like growth of pleomorphic cells within a fibrotic stroma. Pleomorphic cells resembling HL R-S–like cells and lacunar cells. Necrosis frequent	In contrast to HL, CD45+. Positive for CD30 and CD15	Poorly characterized
Plasma Cell Neoplasms			
Monoclonal gammopathy of undetermined significance (MGUS)	Marrow infiltrate with mature plasma cells comprising 1%–9% of cellularity	M-protein < 30 g/L, marrow < 10% plasma cells, no end-organ damage. CD138+. Often difficult to demonstrate LC restriction because of small numbers of plasma cells	Abnormal cytogenetics rarely encountered in MGUS. FISH studies involving *IgH* occur in ~50% of cases: t(11;14), t(4;14). Del13q. Hyperdiploidy 40%
Plasma cell myeloma	Myeloma plasma cells seen in marrow arranged in interstitial clusters	sIg+, CD5-, CD10-, CD19-, CD20-, CD38+(bright), CD45+/-, CD56+, CD117+(bright), CD138+(bright)	IgR, commonly with complex karyotypes and or t(6;14)(p25;q32) involving *MUM1*. t(11;14) seen in 15–25% cases
Extraosseous plasmacytoma	Plasma cells in extraosseous organs must be distinguished from other lymphoproliferative disorders (ie, MALT type)	Same as plasma cell myeloma	Same as above
Solitary plasmacytoma of bone	Plasma cells	Same as plasma cell myeloma	Same as above
Monoclonal immunoglobulin deposition disease	Prominent organ (kidney most common, occasionally liver, heart, nerve, blood vessels involved) deposits of nonamyloid, nonfibrillary, amorphous eosinophilic material that does not stain with congo red. Heavy chain (HCDD) and light chain (LCDD)	LCDD is κ light chain predominant. HCDD shows λ chain predominance. Marrow may show abnormal κ/λ ratio	HCDD with VλVI overrepresentation. LCDD with VκIV variable region
Hodgkin Lymphoma (HL)			
Nodular lymphocyte	"Popcorn cells" with nuclei	BCL6+, CD19+, CD20+,	IgR, with high-level expression

predominant HL (Chap. 59)	resembling those of centroblasts	CD22+, CD45+, CD79a+, CD15-, and rarely CD30+/-, Bob1+, Oct2+, PAX5+	of *BCL6*
Classic HL (Chap. 59)			
Nodular sclerosis HL	R-S cells and lacunar cells dispersed in reactive lymphoid nodules	R-S cells typically are CD15+, CD20-/+, CD30+, CD45-, CD79a-, PAX5+(dim)	R-S cells generally express *PAX5* and *MUM1*, variable expression of *BCL6*, and have IgR without functional Ig
Lymphocyte-rich HL	Few R-S cells with occasional "popcorn" appearance dispersed in lymphoid nodules	Same as above	Same as above
Mixed cellularity HL	R-S cells dispersed among plasma cells, epithelioid histiocytes, eosinophils, and T cells	R-S cells typically are CD15+, CD20-/+, CD30+, CD45-, CD79a-	R-S cells generally express *PAX5* and *MUM1*, variable expression of *BCL6*, and have IgR without functional Ig
Lymphocyte-depleted HL	Prominent numbers of R-S cells with effacement of the nodal structure	Same as above	Same as above
T-Cell Neoplasms			
Immature T-Cell Neoplasms			
Lymphoblastic leukemia (Chap. 54)	Medium to large cells with finely stippled chromatin and scant cytoplasm	TdT+, CD2+/-, cytoplasmic CD3+, CD1a+/-, CD5+/-, CD7+, CD10-/+, CD4+/CD8+ or CD4-/CD8-, CD34+/-	Abnormalities in *TCR* loci at 14q11 (TCR-α), 7q34 (TCR-β), or 7p15 (TCR-γ), and/or t(1;14)(p32-34; q11) involving *TAL1*
Lymphoblastic lymphoma (Chap. 54)	Same as above	Same as above	Same as above
T-Cell Neoplasms			
Mature T- and NK-Cell Neoplasms			
Leukemias			
T-cell prolymphocytic leukemia (Chap. 66)	Small to medium cells with cytoplasmic protrusions or blebs	TdT-, CD2+, CD3+, CD5+, CD7+, CD4+ and CD8- is more common than CD4- and CD8+, but can be CD4+ and CD8+	α/β TCR rearrangement, inv14(q11;q32)(~75%). Inv14 in ~80% of cases. Translocations frequently involve *TCL1A* and *TCL1B* genes. +8q seen in ~75% cases. del 11q23 and abnormalities with chromosome 6 (33%) and 17P (26%) seen
T-cell large granular lymphocytic leukemia (Chap. 57)	Abundant cytoplasm and sparse azurophilic granules	CD2+, CD3+, CD4-/+, CD5+, CD7+, CD8+/-, CD16+/-, CD56-, CD57+/-	α/β TCR rearrangement, γ/δ rearrangement can be seen
Lymphomas/Lymphoproliferative Disorders			
Extranodal T/NK-cell lymphoma, nasal type ("angiocentric lymphoma"; Chaps. 57 and 66)	Angiocentric and angiodestructive growth	CD2+, cytoplasmic CD3+, CD4-, CD5-/+, CD7+, CD8-, CD56+, EBV+	TCR rearrangements usually neg., EBV present by in situ hybridization

Cutaneous T-cell lymphoma (mycosis fungoides; Chap. 65)	Small to large cells with cerebriform nuclei	CD2+, CD3+, CD4+, CD5+, CD7+/-, CD8-, CD25-, CD26+	*α/β* TCR rearrangements, complex karyotype common. STAT3 activation
Sézary syndrome (Chap. 65)	Same as above	Same as above	Same as above
Angioimmunoblastic T-cell lymphoma	Small to medium immunoblasts with clear to pale cytoplasm around follicles and high endothelial venules	CD3+/-, CD4+, CD10+, CXCL13+, PD-1+ (60–100%), EBV+	*α/β* TCR rearrangement (75–90%), IgR (25%–30%), trisomy 3 or 5 noted
Peripheral T-cell lymphoma (not otherwise unspecified; Chap. 66)	Highly variable	CD2+, CD3+, CD5+, CD7-, CD4+CD8- more often than CD4-CD8+, which is more often than CD4+CD8+	*α/β* TCR rearrangement
Subcutaneous panniculitis-like T-cell lymphoma	Variably sized atypical cells with hyperchromasia infiltrating fat lobule	CD2+, CD3+, CD4-, CD5+, CD7-, CD8+, and cytoxic molecules (perforin, granzyme B, and TIA1)	*α/β* TCR rearrangement
Enteropathy associated T-cell lymphoma	Medium to large cells with prominent nucleoli, abundant pale cytoplasm invading mucosal membranes of the small intestine	CD3+, CD5-, CD7+, CD8+/-, CD4-, CD103+, TCR*β*+/-. CD30+ (most cases)	*TRB, TRG* clonally rearranged. >90% *HLADQA1*0501, DQB1*0201*
Hepatosplenic T-cell lymphoma	Small to medium cells with condensed chromatin and round nuclei	CD2+, CD3+, CD4-, CD5+, CD7+/-, CD8+/-	*γ/δ* TCR rearrangement, rarely *α/β* TCR rearrangement, isochromosome 7q
Adult T-cell leukemia/lymphoma (Chap. 54)	Highly pleomorphic with multilobed nuclei	CD2+, CD3+, CD5+, CD7-, CD25+, CD4+CD8- more often than CD4-CD8+	*α/β* TCR rearrangement, integrated HTLV-1
Anaplastic large cell lymphoma ALK-positive	Large pleomorphic cells with “horseshoe”-shaped nuclei, prominent nucleoli, and abundant cytoplasm	TdT-, ALK1+, CD2+/-, CD3-/+, CD4-/+, CD5-/+, CD7+/-, CD8-/+, CD13-/+, CD25+/-, CD30+, CD33-/+, CD45+, HLA-DR+, TIA+/-	TCR rearrangement, t(2;5)(p23;q35) resulting in nucleophosmin–anaplastic lymphoma kinase fusion protein (*NPM/ALK*); other translocations involving 2p23 are also seen
Anaplastic large cell lymphoma ALK-negative	Similar morphologic spectrum to that seen in ALK+ ALCL. No small cell variant seen in ALK–	TdT-, ALK1-, CD2+/-, CD3-/+, CD4-/+, CD5-/+, CD7+/-, CD8-/+, CD13-/+, CD25+/-, CD30+, CD33-/+, CD45+, HLA-DR+, TIA+/-	TCR rearrangement, no recurrent cytogenetic features seen
Primary cutaneous CD30+ anaplastic large cell lymphoma	Anaplastic large cells as above in cutaneous nodules	TdT-, CD2-/+, CD3+/-, CD4+, CD5-/+, CD7+/-, CD25+/-, CD30+, CD45+	TCR rearrangement but without t(2;5) (p23;q35)
Lymphomatoid papulosis	Three histologic subtypes (A, B, C). Type A: scattered clusters of large R-S–like cells admixed with histiocyte rich infiltrate. Type B: rarely seen, epidermotropic infiltrate of small atypical cells with cerebriform nuclei (MF-like). Type C: monotonous large CD30+ T cells with few inflammatory cells	Type A and C have similar phenotype to C-ALCL. Type B phenotype CD3+, CD4+, CD8-, CD30-	TCR rearrangement in 60% cases. No t(2;5)(p23;135)

Primary cutaneous peripheral T-cell lymphomas, rare subtypes:			
Primary cutaneous *γ/δ* T-cell lymphoma	Epidermotropic, dermal and subcutaneous histologic patterns. Neoplastic cells medium to large with coarse chromatin, frequent apoptosis/necrosis	CD3+, CD2+, CD5-, CD7+/-, CD56+. Most cases CD4-, CD8-	*TCRG, TCRD* clonal rearrangement. EBV negative
Primary cutaneous CD8+ aggressive epidermotropic cytotoxic T-cell lymphoma	Variable histology ranging from lichenoid epidermotropism to deeper nodular infiltrates. Tumor cells small to medium with pleomorphic or blastic nuclei	CD3+, CD8+, granzyme B+, perforin+, TIA1+, CD45RA+/-, CD45RO-, CD2+/-, CD4-, CD5-, CD7+/-	Clonal *TCR* rearrangement. EBV negative
T-Cell Neoplasms			
Primary cutaneous CD4+ small/medium T-cell lymphoma	Dense, diffuse, dermal infiltrates. Predominance of small/medium pleomorphic cells	CD3+, CD4+, CD8-, CD30-. No cytotoxic proteins expressed	Clonal *TCR* rearrangement. EBV negative
EBV+ T-cell lymphoproliferative diseases of childhood	Infiltrating T cells are EBV+, but lack cytologic atypia. Erythrophagocytosis and histiocytosis seen frequently	CD2+, CD3+, CD56-, CD8+, EBER+	Clonal TCR rearrangement. EBV+ with LMP1 expression
Hydroa vacciniforme-like lymphoma	Cutaneous presentation, small to medium cells without clear cytology atypical	CD3+, CD8+, CD56+	Clonal TCR rearrangement. EBV+ without LMP1
Natural Killer (NK) Cell Neoplasms			
Large granular lymphocytic leukemia (Chap. 57)	Abundant cytoplasm and sparse azurophilic granules	TdT-, CD2+, CD3-, CD4-, CD5-/+, CD7+, CD8-/+, CD11b+, CD16+, CD56+, CD57+/-	No TCR rearrangement
Aggressive NK-cell leukemia	Same as above	Same as above	No TCR rearrangement, EBV present
Extranodal NK-cell lymphoma, nasal-type (“angiocentric lymphoma”)	Angiocentric and angiodestructive growth	CD2+, cytoplasmic CD3*ε* +, CD4-, CD5-/+, CD7+, CD8-, CD56+	No TCR rearrangement, EBV present
Immunodeficiency-associated lymphoproliferative disorders			
Lymphoproliferative disorders associated with primary immune disorders	Range of morphology from reactive hyperplasia, polymorphous lymphoid infiltrate, to high-grade lymphomas. Lymphoma and HL morphology is similar to that seen in immune competent patients	Immunophenotype similar to that seen in immune competent patients with corresponding malignancy	FAS mutation seen in *ALPS*. Mutations in *SAP/SLAM* in XLP. *ATM* mutations in AT
Lymphomas associated with HIV infection	Similar to above. Typical histologic features seen in Burkitt lymphoma, HL, DLBCL. Lymphomas seen more frequently in HIV setting include primary effusion, plasmablastic, lymphomas, multicentric Castleman disease	Similar to above	*MYC* and *BCL2* translocations seen in DLBCL

Post-transplant lymphoproliferative disorders (PTLDs)			
Early lesions: plasmacytic hyperplasia (PH) and infectious mononucleosis (IM)-like	PH: numerous plasma cells, lymphocytes, and immunoblasts. IM: numerous immunoblasts on a background of T cells	Similar to above	EBV+ in both IM and PH. Oligoclonal polyclonal IgH rearrangement. EBV+
Polymorphic PTLD	Effacement of tissue architecture with infiltrate showing full range of B-cell maturation	Similar to above with exception that R-S cells in HLs often express CD30+, CD20+ but frequently are CD15-	Clonal *IG* rearrangement. EBV+ by EBER ISH. Mutated IgH in 75% of cases
Monomorphic PTLD	Similar to DLBCL, Burkitt lymphoma or plasmacytoma morphology	Similar to above	EBV+/-. Clonal B cell or T cells. Cytogenetics frequently with *TP53, RAS* mutations, *BCL6* translocations
Other iatrogenic immunodeficiency-associated lymphoproliferative disorders	Increased frequency of HL and lymphoproliferation with Hodgkin-like features. Histologic features can otherwise resemble the range of features seen in other immune deficiency-related LPDs	HL-like show CD20+, CD30+, CD15- or CD20-, CD30+, CD15+ staining. EBV is variably positive	Same as above

FISH, fluorescence in situ hybridization; IgR, immunoglobulin gene rearrangement; IgVH, immunoglobulin variable heavy chain; MCD, multicentric Castleman's disease; neg., negative; NF-κB, nuclear factor-κB; NK, natural killer; R-S, Reed-Sternberg; SMZL, splenic marginal zone lymphoma; STAT, signal transducer and activator of transcription; TCR, T-cell receptor. Also see acronyms and abbreviations at the beginning of this chapter.

*The immunophenotype revealed by immunohistochemistry and/or flow cytometry of surface antigens that typically are found for neoplastic cells of a given disorder are listed. If a CD antigen is indicated, then most of the neoplastic cells express that particular surface protein that is expressed by most tumor cells. CD antigens that have a minus (-) sign suffix are characteristically not expressed by the neoplastic cells of that disease entity. CD antigens that have a +/- sign suffix are not expressed by the neoplastic cells of all patients with that entity or are expressed at low or variable levels on the tumor cells. Antigens that have a -/+ sign suffix are expressed at very low levels or by the tumor cells of a minority of patients.

†The common genetic features associated with a given type of neoplasm are indicated. The numbers in parentheses provide the approximate proportion of cases that have the defined phenotype or genetic abnormality.

Source: *Williams Hematology,* 9th ed, Chap. 90, Table 90–1.

TABLE 53–2 INDOLENT LYMPHOMAS

Disseminated lymphomas/leukemias
Chronic lymphocytic leukemia
Hairy cell leukemia
Lymphoplasmacytic lymphoma
Splenic marginal zone B-cell lymphoma (with or without villous lymphocytes)
Plasma cell myeloma/plasmacytoma
Nodal lymphomas
Follicular lymphoma
Nodal marginal zone B-cell lymphoma (with or without monocytoid B cells)
Small lymphocytic lymphoma
Extranodal lymphomas
Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) type

Source: *Williams Hematology*, 9th ed, Chap. 90, Table 90–2.

TABLE 53–3	AGGRESSIVE LYMPHOMAS
Immature B-cell neoplasms	
B-lymphoblastic leukemia/lymphoma	
Mature B-cell neoplasms	
Burkitt lymphoma/Burkitt cell leukemia	
Diffuse large B-cell lymphoma	
Follicular lymphoma grade III	
Mantle cell lymphoma	
Immature T-cell neoplasms	
T-lymphoblastic lymphoma/leukemia	
Peripheral T- and natural killer (NK) cell neoplasms	
T-cell prolymphocytic leukemia/lymphoma	
Aggressive NK cell leukemia/lymphoma	
Adult T-cell lymphoma/leukemia (associated with HTLV-1 [human T-cell leukemia virus type 1])	
Extranodal NK/T-cell lymphoma	
Enteropathy-associated T-cell lymphoma	
Hepatosplenic T-cell lymphoma	
Subcutaneous panniculitis-like T-cell lymphoma	
Peripheral T-cell lymphomas, not otherwise specified	
Angioimmunoblastic T-cell lymphoma	
Anaplastic large cell lymphoma, primary, systemic	
Immune deficiency-associated lymphoproliferative disorders	

Source: *Williams Hematology*, 9th ed, Chap. 90, Table 90–3.

IMPORTANT CLINICAL FEATURES

- These neoplasms range from one of the most indolent malignancies, mucosa-associated lymphoid tissue (MALT) lymphoma (Chap. 63) to one of the most rapidly growing and aggressive human tumors, Burkitt lymphoma (Chap. 64).
- Neoplastic indolent precursor lesions occur in several cases, notably monoclonal B-cell lymphocytosis (Chap. 55), which may undergo clonal evolution to chronic lymphocytic leukemia, and monoclonal gammopathy, which may undergo clonal evolution to lymphoma, macroglobulinemia, myeloma, or amyloidosis (Chap. 67).
- It is now held that myeloma is preceded by monoclonal gammopathy, which is indolent for variable periods of time. Clonal evolution to a progressive B lymphocytic malignancy occurs at a rate of approximately 1% of patients with essential monoclonal gammopathy per year. (See Chaps. 67 and 68.)
- Lymphoid malignancies, particularly acute lymphoblastic leukemia (Chap. 54), lymphoblastic lymphoma (Chap. 54), and Burkitt lymphoma (Chap. 64) are the most likely to lead to tumor lysis syndrome after treatment—something that should be anticipated and prophylactic measures (eg, hydration, allopurinol, or rasburicase) should be utilized.

ASSOCIATED CLINICAL SYNDROMES

Abnormal Production of Immunoglobulin

- Neoplastic B cells can secrete monoclonal immunoglobulin proteins inappropriately (see Chap. 67).

- If the monoclonal protein is IgM, IgA, or some subclasses of IgG (particularly IgG3), it may increase the viscosity of the blood, impairing flow through the microcirculation (see Chaps. 68 and 69) by enhancing erythrocyte–erythrocyte aggregation (pathologic rouleaux).
  — Impaired circulation because of high blood viscosity and pathological rouleaux can result in the "hyperviscosity syndrome" (see Chap. 69).
  — Manifestations of the hyperviscosity syndrome include headache, dizziness, diplopia, stupor, retinal venous engorgement, and coma.
- Monoclonal immunoglobulins also can impair granulocyte or platelet function, or interact with coagulation proteins to impair hemostasis (see Chap. 67).
- Excessive excretion of immunoglobulin light chains can lead to several types of renal tubular dysfunction and renal insufficiency (see Chaps. 67 and 68).
- Cryoglobulins (or immunoglobulins that precipitate at temperatures below 37°C) can result in Raynaud syndrome, skin ulcerations, purpura, or digital infarction and gangrene (see Chaps. 23 and 68).
- Excessive production of certain types of monoclonal immunoglobulin light chains can lead to formation of amyloid (see Chap. 71).
- Production of autoreactive antibodies in relationship to B-lymphocytic neoplasia may lead to:
  — Autoimmune hemolytic anemia (see Chaps. 22 and 23)
  — Autoimmune thrombocytopenia (see Chap. 73)
  — Autoimmune neutropenia (see Chap. 30)
- Autoantibodies directed against tissues are implicated in the etiopathogenesis of diseases such as autoimmune thyroiditis, adrenalitis, encephalitis, or inflammation of other organs.
- Autoantibodies may also be directed against plasma proteins (eg, C1-inhibitor leading to angioedema).
- Autoantibodies may also precede the onset of overt lymphoma.
- Demyelinization can occur in patients with monoclonal immunoglobulin, resulting in peripheral neuropathies, or polyneuropathy (see Chaps. 67, 68, and 69).
- Occasionally, **p**olyneuropathy is associated with **o**rganomegaly, **e**ndocrinopathy, a **m**onoclonal protein, and **s**kin changes, resulting in POEMS syndrome (see Chap. 68).

Marrow and Other Tissue Infiltration

- Neoplastic lymphocytes may infiltrate the marrow extensively, impairing hematopoiesis.
- Neoplastic lymphocyte proliferation or infiltration may result in any combination of splenomegaly and lymphadenopathy of either superficial or deep lymph nodes.
- Neoplastic lymphocytes also can infiltrate extranodal sites:
  — T-cell lymphomas and leukemias frequently involve the skin, mediastinum, or central nervous system.
  — B-cell lymphomas may involve the salivary glands, endocrine glands, joints, heart, lung, kidney, bowel, bone, or other extranodal sites.
  — Marginal zone B-cell lymphoma of MALT frequently involves the stomach, lung, and salivary glands.

Lymphokine-Induced Disorders

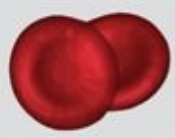
- Neoplastic lymphocytes may elaborate cytokines that contribute to the disease morbidity.
- Cutaneous T-cell lymphomas may elaborate T_H2-type cytokines (eg, interleukins [ILs] 4, 5, 10, and 13), causing eosinophilia or eosinophilic pneumonia (see Chaps. 65 and 66).
- Neoplastic plasma cells may secrete IL-1, a cytokine that can stimulate osteoclasts (leading to extensive osteolysis, severe bone pain, and pathologic fractures) and enhance production of antidiuretic hormone (leading to a syndrome of inappropriate secretion of antidiuretic hormone) (see Chap. 68).
- Dysregulated extrarenal production of calcitriol, the active metabolite of vitamin D, may underlie the hypercalcemia associated with Hodgkin lymphoma and other lymphomas (see Chap. 59).

Systemic Symptoms

- Lymphomas may produce "B symptoms" (eg, fever, night sweats, and weight loss) (see Chaps. 59 and 60).
- Pruritus is common in Hodgkin lymphoma, and its severity parallels disease activity.
- Painful lymphadenopathy after the ingestion of alcohol drinks are a singular feature of Hodgkin lymphoma In a very small fraction of patients (<5%).
- Systemic symptoms may be present in Hodgkin lymphoma in the absence of obvious, bulky lymph node or splenic tumors.

Metabolic Signs

- Aggressive lymphomas and acute lymphocytic leukemias may have high proportions of rapidly dividing and dying cells, causing hyperuricemia and hyperuricosuria.
- Cytotoxic therapy of bulky disease may cause extreme hyperuricemia, hyperuricosuria, hyperkalemia, and hyperphosphatemia, referred to as the *tumor lysis syndrome*.
- Precipitation of uric acid in the renal tubules and collecting system can lead to acute obstructive nephropathy and renal failure.
- Hypercalcemia and calciuria are common complications of plasma cell myeloma.



For a more detailed discussion, see Robert A. Baiocchi: Classification of Malignant Lymphomas, Chap. 90 in *Williams Hematology*, 9th ed.

CHAPTER 54

The Acute Lymphocytic Leukemias

DEFINITION

- Acute lymphocytic (synonym: lymphoblastic) leukemia (ALL) is a neoplastic disease of immature lymphocytes or lymphocyte progenitor cells of either the B- or T-cell lineage.
- The immune phenotype of the leukemia cells reflects the cell lineage and differentiation stage of the transformed clone.
- At diagnosis, the leukemia cells typically have replaced normal cells in the marrow and have disseminated to various extramedullary sites, accounting for many of the clinical manifestations.
- Both immunologic and genetic subgroups have therapeutic implications.
- Survival for patients with ALL has improved with nearly 90% of children and 40% of adults achieving long-term disease-free survival, although patients older than 60 years of age still have a poor prognosis.

ETIOLOGY AND PATHOGENESIS

- Initiation and progression of ALL are driven by successive mutations that alter cellular functions, including an enhanced ability of self-renewal, a subversion of control of normal proliferation, a block in differentiation, and an increased resistance to death signals (apoptosis).
- Ionizing radiation has been implicated as a risk factor for ALL, largely based on the studies conducted at Hiroshima and Nagasaki following the atomic bomb detonations. High birth weight has been associated with an increased risk in the first 5 years of life. Certain congenital (Down syndrome) or inherited abnormalities may increase the risk of later developing ALL. There is ambiguous, inconclusive, or little evidence for other risk factors for this disease (eg, prior chemotherapy, smoking, chemical exposures).

Incidence

- The age-adjusted incidence rate of ALL is 1.6 per 100,000 men and women per year in the United States.
  - — It is estimated that 5320 cases (3140 males and 2180 females) were diagnosed in 2014 in the United States.
  - — The median age at diagnosis for ALL is 13 years, and approximately 61% of cases are diagnosed before the age of 20 years.
  - — ALL is the most common malignancy diagnosed in patients younger than age 15 years,

accounting for 23% of all cancers and 76% of all leukemias in this age group.

- Only 20% of adult acute leukemias have a lymphocytic phenotype.
- Age-specific incidence patterns are characterized by a peak between the ages of 2 and 4 years, followed by falling rates during later childhood, adolescence, and young adulthood (**Figure 54–1**).
- Incidence rises again in the sixth decade.
- The incidence of ALL differs substantially in different geographic areas.
  — Rates are higher among populations in northern and western Europe, North America, and Oceania, with lower rates in Asian and African populations.

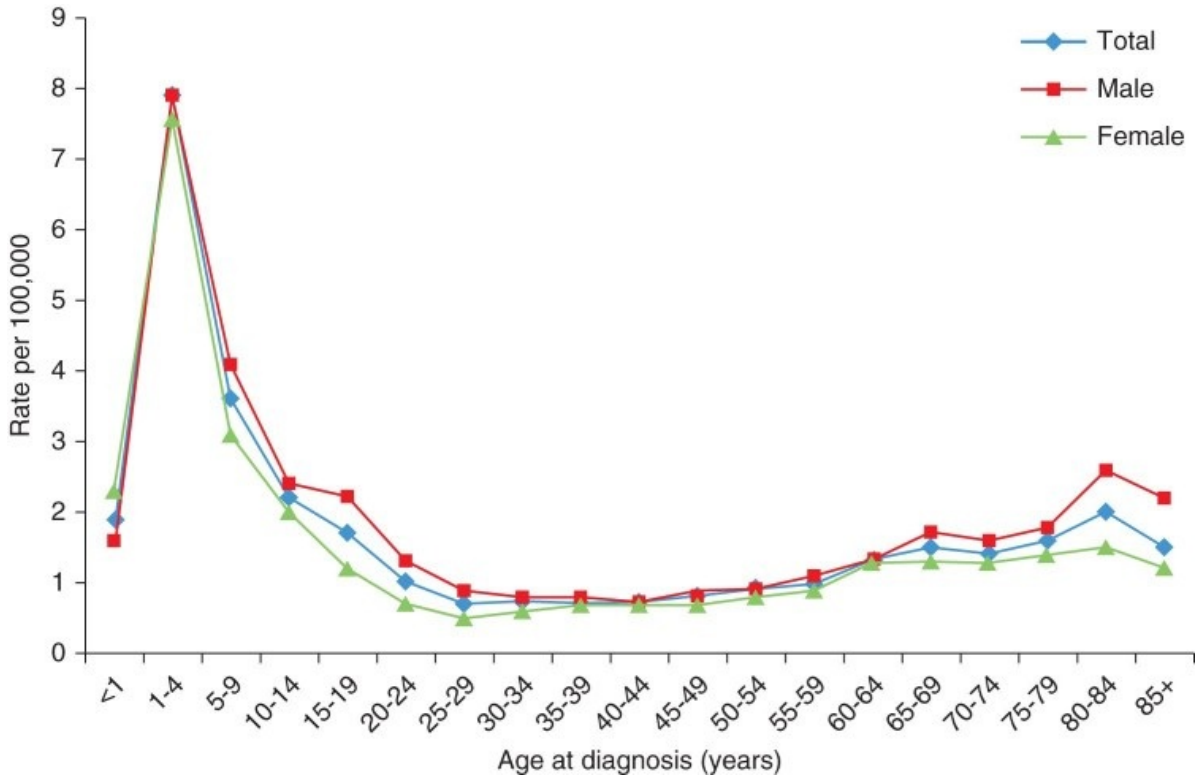


FIGURE 54–1 Age-specific incidence rates for acute lymphoblastic leukemia by sex. (Data from SEER Cancer Statistics Review, 1975–2012, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2012. Accessed January 22, 2016.)

Risk Factors

- Children with Down syndrome have a 10- to 30-fold increased risk of acute leukemias, including ALL.
  — *P2RY8-CRLF2* fusion and activating *JAK* mutations together contribute to leukemogenesis in approximately half of the cases of Down syndrome patients with ALL.
- Patients with genetic syndromes that affect genomic stability and/or DNA repair are at increased risk. These disorders include:
  — Ataxia-telangiectasia
  — Nijmegen breakage syndrome
  — Bloom syndrome
- In utero (but not postnatal) exposure to diagnostic x-rays confers a slightly increased risk of ALL.

Prenatal Origins of Some Cases

- Retrospective identification of leukemia-specific fusion genes (eg, *MLL-AF4*, *ETV6-RUNX1* [also known as *TEL-AML1*]) and development of concordant leukemia in identical twins indicate that some leukemias have a prenatal origin.
  - — The coincidence of ALL among identical twins is about 20% overall, but approaches 100% if the index twin had developed ALL with a t(4, 11)/KMTZA/AFFI and the latency period is short.
  - — The lower concordance rate in twins with the *ETV6-RUNX1* fusion or T-cell phenotype and the longer postnatal latency period suggest additional postnatal events are required for leukemic transformation in this subtype.

Acquired Genetic Changes

- Approximately 80% of all cases have a cytogenetic abnormality or molecular lesions.
- The frequency of specific cytogenetic or molecular abnormalities varies between children and adults as shown in **Table 54–1**.
- Hyperdiploidy (> 50 chromosomes), which occurs in approximately 33% of pediatric cases and in 6% of adult cases, is associated with a more favorable prognosis.
- Hypodiploidy (< 45 chromosomes) is associated with a poor prognosis.
- The most commonly recognized structural abnormalities result from translocations, followed by inversions, deletions, point mutations, and amplifications.
- Cooperating mutations are necessary for leukemic transformation and include genetic and epigenetic changes in key growth factor regulatory pathways.
- In one study, more than 40% of B-cell precursor ALL cases had mutations in genes encoding regulators of normal lymphoid development.
- The most frequent target was the lymphoid transcription factor *PAX5* (mutated in approximately 30% of cases), which encodes a paired-domain protein required for the pro-B-cell to pre-B-cell transition and B-lineage fidelity.
  - — The second most frequently involved gene was *IKZF1* (mutated in almost 30% of the cases), encoding the IKAROS zinc finger DNA-binding protein that is required for the earliest lymphoid differentiation.
  - — In approximately half of *BCR-ABL1* ALL cases, the CRLF2 gene is overexpressed.
- Overexpression of FLT3, a receptor tyrosine kinase important for development of hematopoietic stem cells, is a secondary event in almost all cases with either *MLL* rearrangements or hyperdiploidy.
- Epigenetic changes are important and methylation of several genes is associated with a poor outcome.

TABLE 54–1 FREQUENCIES OF COMMON GENETIC ABERRATIONS IN CHILDHOOD AND ADULT LYMPHOBLASTIC LEUKEMIA

Abnormality	Children (%)	Adults (%)
Hyperdiploidy (> 50 chromosomes)	23–29	6–7
Hypodiploidy (< 45 chromosomes)	1	2
t(1;19)(q23;p13.3) *[TCF3-PBX1]*	4 in white, 12 in black	2–3
t(9;22)(q34;q11.2) *[BCR-ABL1]*	2–3	25–30

t(4;11)(q21;q23) *[MLL-AF4]*	2	3–7
t(8;14)(q23;q32.3)	2	4
t(12;21)(p13;q22) *[ETV6-RUNX1]*	20–25	0–3
NOTCH1 mutations*	7	15
HOX11L2 overexpression*	20	13
LYL1 overexpression*	9	15
TAL1 overexpression*	15	3
HOX11 overexpression*	7	30
MLL-ENL fusion	2	3
Abnormal 9p	7–11	6–30
Abnormal 12p	7–9	4–6
del(7p)/del(7q)/monosomy 7	4	6–11
+8	2	10–12
Intrachromosomal amplification of chromosome 21 (iAMP21)	2	?

*Abnormalities found in T-cell acute lymphoblastic leukemia.
Source: *Williams Hematology*, 9th ed, Chap. 91, Table 91–1.

CLINICAL FEATURES

- Symptoms may appear insidiously or acutely and presenting features generally reflect the degree of marrow failure and the extent of extramedullary spread.
- **Table 54–2** compares the presenting manifestations of ALL in children and adults.
- About half of the patients present with fever that is induced by pyrogenic cytokines (interleukin [IL]-1, IL-6, and tumor necrosis factor) released from leukemia cells; this typically resolves within 72 hours after the start of induction chemotherapy.
- More than 8% to 10% of childhood cases and 15% of adult cases present with an anterior mediastinal mass that, in some cases, can compress the great vessels and trachea sufficiently to cause the superior mediastinal syndrome.
- More than 25% of pediatric cases present with bone or joint pain secondary to leukemia-cell infiltration or marrow necrosis.
- Infiltration of the testicle or lymphatic obstruction by leukemia cells can cause painless enlargement of the scrotum.
- Less common signs and symptoms include those resulting from leukemia-cell involvement of the central nervous system (CNS) (eg, headache, vomiting, alteration of mental function), renal collecting system (oliguria, anuria), eyes (diplopia, visual loss), salivary glands (Mikulicz syndrome), peripheral nerves (cranial nerve palsy), skin (leukemia cutis), dorsal vein, and sacral nerves (priapism).
- In rare cases, the patient may present with spinal cord compression caused by epidural leukemia-cell mass.

TABLE 54–2 **PRESENTING CLINICAL FEATURES IN CHILDREN AND ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA**

Feature	Children (%)	Adult (%)
Age (years)		
< 1	2	—
1–9	72–78	—
10–19	20–26	—
20–39	—	40
40–59	—	40
≥ 60	—	20
Male	56–57	62
Symptoms		
Fever	57	33–56
Fatigue	50	Common
Bleeding	43	33
Bone or joint pain	25	25
Lymphadenopathy		
None	30	51
Marked (> 3 cm)	15	11
Hepatomegaly		
None	34	65
Marked (below umbilicus)	17	Rare
Splenomegaly		
None	41	56
Marked (below umbilicus)	17	Uncommon
Mediastinal mass	8–10	15
Central nervous system leukemia	3	8
Testicular leukemia	1	0.3

Data from Pui CH: Acute lymphoblastic leukemia, in *Childhood Leukemias,* 2nd ed, edited by CH Pui, p. 439. Cambridge University Press, New York, 2006 and Larson RA, Dodge RK, Burns CP, et al: A five-drug remission induction regimen with intensive consolidation for adults with acute lymphoblastic leukemia: Cancer and Leukemia Group B study 8811. Blood 85:2025, 1995.

LABORATORY FEATURES

- The comparison of the presenting laboratory features of ALL in childhood and adult patients is shown in **Table 54–3**.
- Anemia, neutropenia, and thrombocytopenia are common findings at presentation.
- Severity reflects the degree of marrow replacement by leukemic lymphoblasts.
- Approximately 30% present with blood neutrophil counts of less than ($< 0.5 \times 10^9$/L).
- Patients can present with blood leukocyte counts ranging from 0.1 to 1500×10^9/L, and more than 10% of patients will have leukocyte counts greater than ($> 100 \times 10^9$/L) composed principally of lymphoblasts.
- Approximately 10% of patients, usually with striking leukopenia, do not have blasts in the

blood film at the time of diagnosis.

- Findings that may precede the diagnosis of ALL by one to several months include:
  — Pancytopenia simulating aplastic anemia (often with spontaneous recovery)
  — In rare cases, hypereosinophilic syndrome (eg, pulmonary infiltration, cardiomegaly, and congestive heart failure), particularly in males who have ALL cells with t(5;14)(q31;q32)
- Activation of the IL-5 gene on chromosome 5 by the enhancer element of the immunoglobulin heavy chain gene on chromosome 14 is thought to play a central role in leukemogenesis and the associated eosinophilia with t(5;14)(q31;q32).
- Occasional patients, principally male, present with thrombocytosis (> 400 × 10^9/L).
- Serum lactic acid dehydrogenase is elevated in most patients, and the level correlates with tumor burden.
- Increased levels of serum uric acid are common in patients with a large leukemic burden.
- Patients with exaggerated hyperuricemia may have spontaneous tumor lysis syndrome before therapy with associated increased levels of creatinine, urea nitrogen, and phosphorus.
- Less common laboratory abnormalities include hypercalcemia (caused by release of a parathyroid-like hormone from leukemia blasts), elevated serum transaminases (caused by liver infiltration), or azotemia (caused by renal failure secondary to kidney infiltration).
- Less common t(17;19)(q22;13.3) with *E2A-HLF* fusion, found in 0.5% of B-cell precursor ALL, is associated with:
  — Adolescent age
  — Disseminated coagulopathy
  — Hypercalcemia
  — Dismal prognosis
- Mediastinal mass and enlargement of the thymus may be seen on chest radiograph.
- About half of all pediatric cases present with periosteal reactions, osteolysis, osteosclerosis, or osteopenia, especially those with low leukocyte counts at presentation.

TABLE 54–3 PRESENTING LABORATORY FEATURES IN CHILDREN AND ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

	Percent of Total	
Feature	**Children, White/Black (%)**	**Adults (%)**
Cell lineage		
T cell	15/24	25
B-cell precursor	85/76	75
Leukocyte count (× 10^9/L)		
< 10	47–49/34	41
10–49	28–31/29	31
50–99	8–12/14	12
> 100	11–13/23	16
Hemoglobin concentration (g/dL)		
< 8	48/58	28
8–10	24/22	26
> 10	28/20	46

Platelet count ($\times 10^9$/L)		
< 50	46/40	52
50–100	23/20	22
> 100	31/40	26
CNS status*		
CNS1	67–79/60	92–95
CNS2	5–24/27	?
CNS3	3/3	5–8
Traumatic lumbar puncture with blasts	6–7/10	?
Leukemic blasts in marrow (%)		
< 90	33/46	29
> 90	67/54	71
Leukemic blasts in blood		
Present	87/90	92
Absent	13/10	8

*CNS1, no blast cells in cerebrospinal fluid sample; CNS2, < 5 leukocytes/μL with blast cells in a nontraumatic sample; CNS3, ≥ 5 leukocytes/μL with blast cells in a nontraumatic sample or the presence of a cranial nerve palsy; and traumatic lumbar puncture with blasts (≥ 10 erythrocytes/μL with blasts). Data on CNS2 and traumatic lumbar puncture with blasts are not available in adults. Data from in Pui CH: Acute lymphoblastic leukemia, in *Childhood Leukemias*, 2nd ed, edited by CH Pui, p. 439. Cambridge University Press, New York, 2006 and Larson RA, Dodge RK, Burns CP, et al: A five-drug remission induction regimen with intensive consolidation for adults with acute lymphoblastic leukemia: Cancer and Leukemia Group B study 8811. Blood 85:2025, 1995.

Evaluating CNS Disease

- It is essential to evaluate the cerebrospinal fluid (CSF) at diagnosis because of the high rate of CNS involvement.
  - — CNS leukemia is defined by the presence of at least five leukocytes per microliter of CSF or by the presence of cranial nerve palsies.
  - — The presence of any leukemic blast cells in the CSF is associated with increased risk of CNS relapse.
- Contamination of the CSF by leukemic cells as a result of traumatic lumbar puncture at diagnosis is associated with an inferior treatment outcome in children with ALL.
  - — Risk of traumatic lumbar puncture can be decreased by administering platelet transfusions to thrombocytopenic patients and by having the most experienced clinician perform the procedure after the patient is under deep sedation or general anesthesia.

Diagnosis and Cell Classification

- Examination of a marrow aspirate is preferable for the diagnosis of ALL.
- B-cell blasts in ALL are characterized by intensely basophilic cytoplasm, regular cellular features, prominent nucleoli, and cytoplasmic vacuolation (**Figure 54–2**).
- Analysis of a Wright-Giemsa stain (eg, blood or marrow film) is insufficient to differentiate ALL from acute myeloid leukemia with certainty.
- Immunophenotyping of leukemia cells can help distinguish immunologic subtypes of ALL.

- A distinct subset of T-cell ALL that retain stem cell-like features, termed *early T-cell precursor ALL*, has been identified that is associated with a dire prognosis with conventional chemotherapy.
- **Table 54–4** summarizes the salient presenting features of several recognized immunologic subtypes of ALL.
- Genetic classification
  — Approximately 75% of adult and childhood cases can be readily classified into prognostically or therapeutically relevant subgroups based on the modal chromosome number (or DNA content estimated by flow cytometry), specific chromosomal rearrangements, and molecular genetic changes.
  — Two ploidy groups (hyperdiploidy > 50 chromosomes and hypodiploidy < 45 chromosomes) have clinical relevance. Hyperdiploidy, which is seen in approximately 25% of childhood cases and in 6% to 7% of adult cases, is associated with a favorable prognosis.
- Phenotype-specific reciprocal translocations are the most biologically and clinically significant karyotypic changes in ALL (**Table 54–5**).

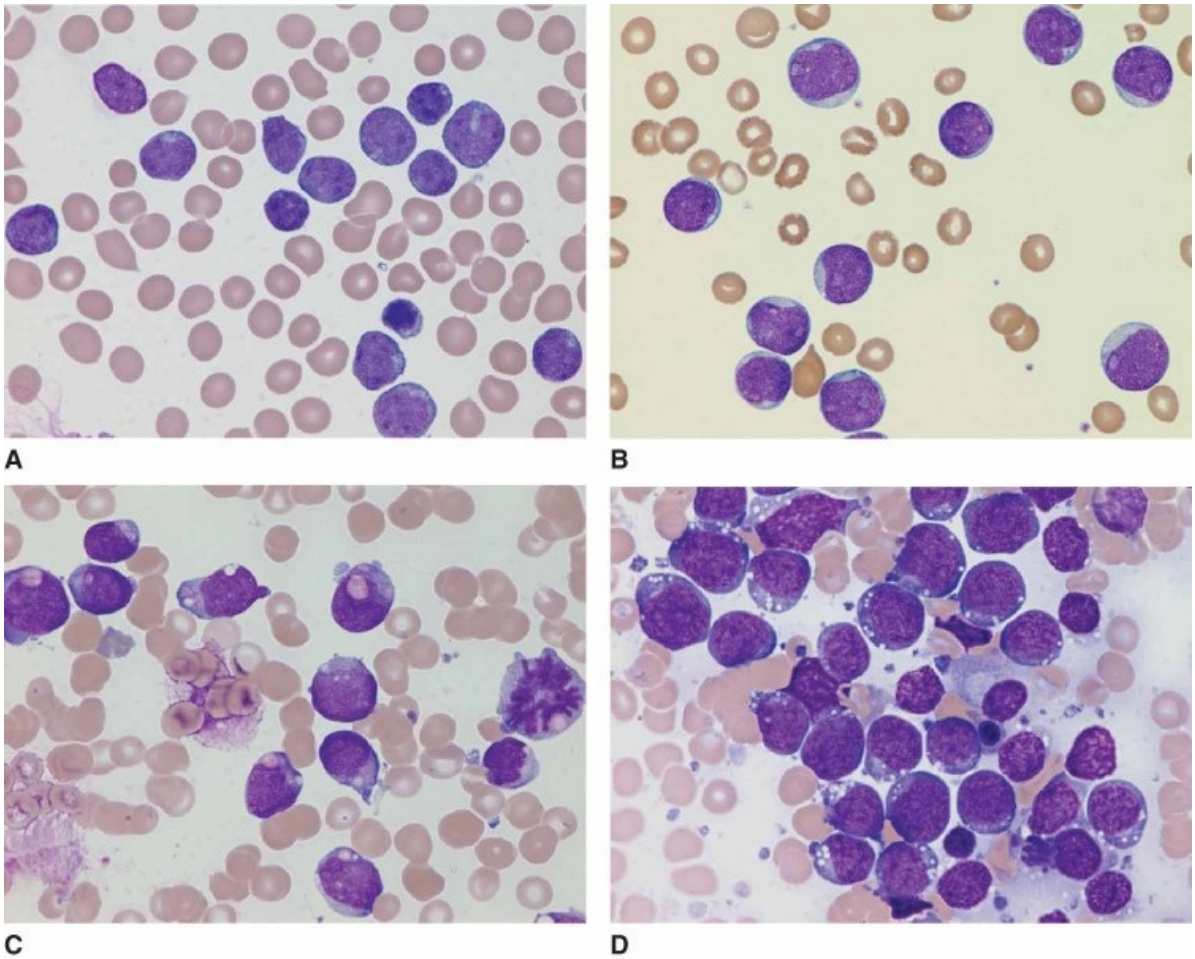


FIGURE 54–2 **A.** Typical lymphoblasts with scanty cytoplasm, regular nuclear shape, fine chromatin, and indistinct nucleoli. **B.** Acute lymphoblastic leukemia (ALL) with large blasts showing prominent nucleoli, moderate amounts of cytoplasm, and an admixture of smaller blasts. **C.** ALL with cytoplasmic granules. Fuchsia granules are present in the cytoplasm of many blasts. Such granules may lead to a misdiagnosis of acute myeloid leukemia; however, the granules are negative for myeloperoxidase and myeloid-pattern Sudan black B staining. **D.** B-cell ALL lymphoblasts. The blasts in this phenotype are characterized by intensely basophilic cytoplasm, regular cellular features, and cytoplasmic vacuolation. (Images **A** to **D,** Wright-Giemsa stain; original magnification ×1000.) Source: *Williams Hematology*, 9th ed, Chap. 91, Fig. 91–4.

TABLE 54–4 PRESENTING FEATURES OF ACUTE LYMPHOBLASTIC LEUKEMIA ACCORDING TO IMMUNOLOGIC SUBTYPE

Subtype	Typical Markers	Childhood (%)	Adult (%)	Associated Features
B-cell precursor	CD19+, CD22+, CD79a+, cIg+/–, sIgμ–, HLA-DR+			
Pro-B	CD10–	5	11	Infants and adult age group, high leukocyte count, initial CNS leukemia, pseudodiploidy, *MLL* rearrangement, unfavorable prognosis
Early pre-B	CD10+	63	52	Favorable age group (1–9 years), low leukocyte count, hyperdiploidy (> 50 chromosomes)
Pre-B	CD10+/–, cIg+	16	9	High leukocyte count, black race, pseudodiploidy
Mature B cell (Burkitt)	CD19+, CD22+, CD79a+, cIg+, sIg+ (kappa or lambda+)	3	4	Male predominance, initial CNS leukemia, abdominal masses, often renal involvement
T lineage	CD7+, cCD3+			
T cell	CD2+, CD1+/–, CD4+/–, CD8+/–, HLA-DR–, TdT+/–	10	18	Male predominance, hyperleukocytosis, extramedullary disease
Pre-T	CD2–, CD1–, CD4–, CD8–, HLA-DR+/–, TdT+	1	6	Male predominance, hyperleukocytosis, extramedullary disease, unfavorable prognosis
Early T-cell precursor	CD1–, CD8–, CD5^{weak}, CD13+, CD33+, CD11b+, CD117+, CD65+, HLA-DR+	2	?	Male predominance, age >10 years, poor prognosis

cCD3, cytoplasmic CD3; cIg, cytoplasmic immunoglobulin; sIg, surface immunoglobulin; TdT, terminal deoxynucleotidyl transferase.
Source: *Williams Hematology*, 9th ed, Chap. 91, Table 91–4.

TABLE 54–5 CLINICAL AND BIOLOGIC FEATURES ASSOCIATED WITH THE MOST COMMON GENETIC SUBTYPES OF ACUTE LYMPHOBLASTIC LEUKEMIA

		Estimated Event-Free Survival (%)	
Subtype	Associated Features	Children	Adults
Hyperdiploidy (> 50 chromosomes)	Predominant precursor B-cell phenotype; low leukocyte count; favorable age group (1–9 years) and prognosis in children	80–90 at 5 years	30–50 at 5 years
Hypodiploidy (< 45 chromosomes)	Predominant precursor B-cell phenotype; increased leukocyte count; poor prognosis	30–40 at 3 years	10–20 at 3 years
t(12;21)(p13;q22) *[ETV6-RUNX1]*	CD13+/–CD33+/– precursor B-cell phenotype; pseudodiploidy; age 1–9 years; favorable prognosis	90–95 at 5 years	Unknown
t(1;19)(q23;p13.3) *[TCF3-PBX1]*	CD10+/–CD20–CD34– pre-B phenotype; pseudodiploidy; increased leukocyte count; black race; CNS leukemia; prognosis depends on treatment	82–90 at 5 years	20–40 at 3 years

t(9;22)(q34;q11.2) *[BCR-ABL1]*	Predominant precursor B-cell phenotype; older age; increased leukocyte count; myeloid antigens; improved early outcome with tyrosine kinase inhibitor treatment	80–90 at 3 years	~60 at 1 year
t(4;11)(q21;23) with *MLL-AF4* fusion	CD10+/–CD15+/–CD33+/–CD65+/– precursor B-cell phenotype; infant and older adult age groups; hyperleukocytosis; CNS leukemia; poor outcome	32–40 at 5 years	10–20 at 3 years
t(8;14)(q24;q32.3)	Mature B-cell phenotype; L3 morphology; male predominance; bulky extramedullary disease; favorable prognosis with short-term intensive chemotherapy including high-dose methotrexate, cytarabine, and cyclophosphamide/ifosfamide	75–85 at 5 years	70–80 at 4 years
NOTCH 1 mutations	T-cell phenotype; favorable prognosis	90 at 5 years	50 at 4 years
HOX11 overexpression	CD10+ T-cell phenotype; favorable prognosis with chemotherapy alone	90 at 5 years	80 at 3 years
Intrachromosomal amplification of chromosome 21	Precursor B-cell phenotype; low white blood cell count; intensified treatment required to avert a poor prognosis	30 at 5 years	?

Data from Pui CH, Robison LL, Look AT: Acute lymphoblastic leukemia. *Lancet* 371:1030, 2008; Schultz KR, Bow-man WP, Aledo A, et al: Improved early event free survival with imatinib in Philadelphia chromosome-positive acute lymphoblastic leukemia: A Children's Oncology Group Study. *J Clin Oncol* 27:5715, 2009; Larson RA, Dodge RK, Burns CP, et al: A five-drug remission induction regimen with intensive consolidation for adults with acute lymphoblastic leukemia: Cancer and Leukemia Group B study 8811. *Blood* 85:2025, 1995; Rizzieri DA, Johnson JL, Byrd JC, et al; Alliance for Clinical Trials In Oncology (ACTION). Improved efficacy using rituximab and brief duration, high intensity chemotherapy with filgrastim support for Burkitt or aggressive lymphomas: Cancer and Leukemia Group B study 10002. *Br J Haematol* 165(1):102–111, 2014.

DIFFERENTIAL DIAGNOSIS

- ALL should be considered in the differential diagnosis of patients with hypereosinophilia.
- ALL should be considered in the differential diagnosis of children and young adults with apparent aplastic anemia. Rarely, ALL may appear after a short period of disease simulating aplastic anemia.
- Occasionally, hematogones in a regenerating marrow may mimic leukemic blast cells and require flow cytometry examination with optimal combinations of antibodies to distinguish these cells from residual leukemia cells.
- A major consideration in the differential diagnosis is whether the leukemic blasts represent those of acute myelogenous leukemia (see Chap. 45).
- Marrow infiltration by small round-cell nonhematopoietic tumors (eg, neuroblastoma, rhabdomyosarcoma, Ewing sarcoma, small cell lung cancer) (see Chap. 12).
- Infectious diseases (eg, mononucleosis especially those associated with thrombocytopenia) or hemolytic anemia (see Chap. 23) or pertussis (see Chap. 49).

TREATMENT

Metabolic Complications

- Hyperuricemia can be treated with allopurinol (300 mg/d) or rasburicase (recombinant urate oxidase).

— Allopurinol can decrease both the anabolism and catabolism of mercaptopurine by depleting intracellular phosphoribosyl pyrophosphate and by inhibiting xanthine oxidase.
— If mercaptopurine and allopurinol are given together orally, the dosage of mercaptopurine generally must be reduced.
— Rasburicase acts more rapidly than allopurinol and breaks down uric acid to allantoin, a readily excreted metabolite that is five to ten times more soluble than uric acid. However, rasburicase is contraindicated in patients with glucose-6-dehydrogenase deficiency because hydrogen peroxide, a by-product of uric acid breakdown, can cause methemoglobinemia or hemolytic anemia.

- Hyperphosphatemia can be treated with a phosphate binder (eg, aluminum hydroxide, calcium carbonate or acetate).

Hyperleukocytosis

- Hyperleukocytosis (> 400×10^9 white cells/L) is treated with
  — Either leukapheresis or exchange transfusions (in small children)
  — Preinduction therapy with low-dose glucocorticoids, with addition of vincristine and cyclophosphamide in cases of B-cell ALL—a favored means of ameliorating hyperleukocytosis used in conjunction with urate oxidase

Infection Control

- Exercise precautions should be taken in immunocompromised persons (eg, avoidance of overtly infected persons, uncooked vegetables, unpeeled fruits, raw cheese).
- Broad-spectrum antibiotics may be given to febrile patients with newly diagnosed ALL, especially in the setting of neutropenia.
- Trimethoprim-sulfamethoxazole should be considered for prophylaxis against pneumonia with *Pneumocystis jiroveci*.
- Prophylaxis is started 2 weeks after remission induction and continues until 6 weeks after completion of all chemotherapy.
- Alternative treatments for patients who cannot tolerate trimethoprim-sulfamethoxazole include aerosolized pentamidine and atovaquone (which should be taken with food or a milky drink).
- Antifungal prophylaxis is commonly given.
- Live-virus vaccines should not be administered.

Hematologic Support

- Platelet transfusions should be given for platelet counts less than 10×10^9/L or for thrombocytopenic bleeding.
- Red cell transfusions should be given slowly in patients with profound anemia.
- All blood products should be irradiated.

Antileukemic Therapy

- There is no standard therapy for all patients with ALL and treatment is increasingly targeted to biologically distinct subtypes.

B-Cell ALL (Burkitt type)

- Aggressive cyclophosphamide-based regimens administered for 3 to 6 months are most effective for patients with B-cell ALL (L3 morphology, surface immunoglobulin-positive).
  — Effective CNS therapy is an essential component of successful regimens for B-cell ALL and generally consists of methotrexate and cytarabine administered both systematically and intrathecally.
  — B-cell ALL rarely, if ever, recurs after the first year; therefore, prolonged continuation therapy is not necessary.

Precursor B-Cell ALL and T-Cell ALL

- There are typically three standard phases of treatment for the average-risk patient, both children and adults:
  — Remission induction
  — Consolidation
  — Maintenance (continuation) therapy

Remission Induction

- Goal is to profoundly decrease the body burden of leukemia cells to prevent further spread of the disease to the brain and spinal cord.
- Hospitalization is usually necessary at some point to help prevent infection and to administer blood products.
- Specific drugs vary and are different for adults and children.
- For induction, nearly everyone receives vincristine and a glucocorticoid (eg, prednisone, prednisolone, or dexamethasone), and adults typically also receive an anthracycline, such as daunorubicin.
- Adding L-asparaginase to the induction regimen provides for response rates of approximately 98% in children and approaching 90% in adults with standard-risk disease.
- There are three forms of L-asparaginase, each with a different pharmacokinetic profile when given intravenously:
  — *Erwinia carotovora* L-asparaginase has a relatively short half-life.
  — *Escherichia coli* L-asparaginase may be useful.
  — *Polyethylene glycol (PEG)* L-asparaginase (pegaspargase) has the longest half-life.
  — The dose intensity and duration of treatment are more important than the type of L-asparaginase used.
- Because of lower immunogenicity, improved efficacy, and less frequent administration, pegaspargase has replaced the native product as the first-line treatment for children in the United States.
- The amount of residual leukemia identified using molecular or immunologic techniques after remission has been achieved correlates with long-term outcome.

Consolidation Therapy

- This regimen is defined as treatment given shortly after remission induction on restoration of normal hematopoiesis.

- Regimens typically consist of high doses of multiple agents not used during induction therapy or readministration of the induction regimen.
- Consolidation therapy has improved outcome in pediatric patients, even in those with low-risk disease.
- Commonly used regimens in childhood cases include:
  — High-dose methotrexate (5 g/m^2), which is particularly important in T-cell ALL with or without 6-mercaptopurine
  — High-dose L-asparaginase given for an extended period
  — Combinations of dexamethasone, vincristine, L-asparaginase, doxorubicin, cytarabine, and thioguanine, with or without cyclophosphamide
- Consolidation therapy for adult patients with ALL has become standard.
  — Consolidation regimens similar to those used in children should be given to adults up to age 39 years.
  — In adults, methotrexate dose probably should be limited to 1.5 to 2 g/m^2 because higher doses may lead to excessive toxicities.

Maintenance or Continuation Therapy

- Maintenance, or continuation, therapy usually involves daily low-dose chemotherapy given for 2 to 3 years.
  — Because boys, but not girls, benefit from a third year of therapy, girls typically receive 2 to 2.5 years and boys 3 years of maintenance therapy.
  — In most adult trials, maintenance therapy is given for 2 years.
- Mature B-cell ALL rarely recurs after the first year, so prolonged maintenance therapy is not recommended.
- Maintenance therapy typically involves weekly administration of methotrexate (orally or intravenously) and daily doses of oral 6-mercaptopurine to patients who remain in remission.
- Intermittent pulses of vincristine and a glucocorticoid may improve the efficacy of such antimetabolite-based regimens.
- Studies show that maintenance therapy lowers the relapse rate in childhood ALL, except in cases of mature B-cell leukemia.
- Mercaptopurine is best given in the evening and should not be given with milk or milk products.
- One in 300 patients have an inherited deficiency of thiopurine *S*-methyltransferase, are highly sensitive to 6-mercaptopurine, and can tolerate only small amounts of drug.
- Reinduction therapy early in first remission is used increasingly.

Treatment of CNS

- Extra treatment measures typically are necessary for eradicating leukemia cells from sanctuary disease sites (eg, brain, testes, and spinal cord) that do not achieve therapeutic drug concentrations during systemic chemotherapy.
- The high rate of CNS relapse after successful systemic chemotherapy can be lowered to as low as 2% to 4% with prophylactic therapy of the CNS.
- Intrathecal (IT) chemotherapy may be as effective as cranial irradiation:

— IT methotrexate for low-risk and intermediate-risk ALL
— Combined IT methotrexate, hydrocortisone, and cytosine arabinoside for high-risk ALL
- CNS relapse requires systemic therapy along with treatment of CNS (cranial irradiation 24 Gy plus combined triple IT therapy).
- The outcome after CNS relapse is poor, analogous to the outcome after marrow relapse.
- Survival after CNS relapse is usually less than 1 year in adults.

Allogeneic Hematopoietic Stem Cell Transplantation

- This treatment is controversial for patients during first remission.
- It is typically considered for patients who have histocompatible donors and who:
  — Require extended (>4 weeks) of induction therapy because of refractory disease
  — Are adults and have high leukocyte counts on presentation or t(11; 14)
  — Develop hematologic relapse while on therapy or shortly thereafter
  — Have a high level of minimal residual disease after remission

BCR-ABL–Positive ALL

- The use of tyrosine kinase inhibitors imatinib or dasatinib in may be effective in BCR-ABL–positive ALL.
- In combination with chemotherapy, the tyrosine kinase inhibitor not only induced a higher complete remission rate but also a higher rate of molecular remission in adults.
- Rituximab improves outcome in patients with B-cell precursor ALL whose lymphoblasts express CD-20.
- Nelarabine is active in T-cell ALL.

COURSE AND PROGNOSIS

Relapse

- Relapse of disease may occur at any site.
- Most relapses occur during treatment or within the first 2 years after its completion.
- Factors that influence the likelihood of relapse include:
  — Age over 30 years
  — A high white blood cell count at the time of diagnosis (eg, > 50×10^9/L)
  — Disease that has spread beyond the marrow to other organs
  — Certain genetic abnormalities, such as *MLL* gene rearrangements (at 11q23)
  — The need for extended (> 4 weeks) induction therapy to attain complete remission
- Poor prognosis after relapse:
  — Relapse while on therapy or after a short initial remission
  — T-cell ALL immunophenotype
  — The presence of the *BCR-ABL* translocation
  — An isolated hematologic relapse
  — Presence of minimal residual disease after reinduction treatment
  — Adults with isolated CNS relapse
- Marrow relapse is the most common site of relapse and portends a poor outcome.

- Typical signs or symptoms are anemia, leukocytosis, leukopenia, thrombocytopenia, bone pain, fever, or sudden decrease in tolerance to chemotherapy.
- Prolonged second remissions (> 3 years) may be obtained with chemotherapy in about 50% of patients with a late relapse (eg, > 6 months after cessation of therapy), but only in about 10% of those with an early relapse.
- In most patients, the best chance for cure is an allogeneic transplant. A small fraction of patients who relapse after an allogeneic transplant can be cured with a second transplant.
- Testicular relapse:
  — One-third of patients with early testicular relapse and two-thirds of patients with late testicular recurrence became long-term survivors after salvage chemotherapy and testicular irradiation.
  — Late relapse after cessation of maintenance is compatible with subsequent long disease-free survival after treatment.
  — Treatment recommended is bilateral testicular irradiation and systemic reinduction therapy.
- CNS relapses:
  — Efficacy of salvage therapy depends on history of prior CNS irradiation.
  — Intensive chemotherapy and craniospinal irradiation can secure long-term remission in previously unirradiated patients.

Treatment Sequelae

- Currently, the induction mortality ranges between 2% and 11% in adult ALL, but is less than 2% in children.
- A major source of mortality is infection with bacteria or fungi.
- The death rate among elderly patients receiving remission induction therapy can be as high as 30%.
- Poor tolerance of chemotherapy and consequent reduction of dose intensity largely account for the generally poor clinical outcome in elderly patients.
- **Table 54–6** lists the side effects associated with drugs used in therapy.
- Potential acute side effects of therapy occurring during or shortly after induction therapy:
  — Hyperglycemia with glucocorticoid use in more than 10% of cases
  — Pancreatitis in a subset of patients treated with L-asparaginase
  — Cerebral venous sinus thrombosis, which may occur in 2% of children treated with L-asparaginase.
  — Mucositis with anthracycline or antimetabolite chemotherapy
  — Tumor lysis syndrome
  — Hypercoagulable state
  — Complications of marrow suppression
- Potential delayed side effects of therapy include the following:
  — Neurologic impairment from CNS therapy (less without radiotherapy)
  — Growth and development impairment
  — Aseptic necrosis of the bone
  — Obesity, which occurs in 30% of young adult survivors of ALL
  — Testicular damage in boys
- There is a risk of development of a second malignancy.

— Brain tumors and acute myelogenous leukemia are most common.
— Median latency period is 9 to 20 years, depending on the type of second malignancy.

TABLE 54–6 SIDE EFFECTS ASSOCIATED WITH ANTILEUKEMIC THERAPY

Treatment	Acute Complications	Late Complications
Prednisone (or prednisolone)	Hyperglycemia, hypertension, changes in mood or behavior, acne, increased appetite, weight gain, peptic ulcer, hepatomegaly, myopathy	Avascular necrosis of bone, osteopenia, growth retardation
Dexamethasone	Same as prednisone, except for increased changes in mood or behavior and myopathy, but less salt retention	Same as prednisone
Vincristine	Peripheral neuropathy, constipation, chemical cellulitis, jaw pain, seizures, hair loss	
Daunorubicin, idarubicin, doxorubicin, or epirubicin	Nausea and vomiting, hair loss, mucositis, marrow suppression, chemical cellulitis, increased skin pigmentation	Cardiomyopathy (after high cumulative dose)
L-Asparaginase	Nausea and vomiting, allergic reactions (manifested as rashes, bronchospasm, severe pain at intramuscular injection site), hyperglycemia, pancreatitis, liver dysfunction, large vein thrombosis, encephalopathy	None
Mercaptopurine	Nausea and vomiting, mucositis, marrow suppression, solar dermatitis, liver dysfunction: increased hematologic toxicity in persons lacking thiopurine methyltransferase	Osteoporosis (long-term use), acute myeloid leukemia in persons with thiopurine methyltransferase deficiency
Methotrexate	Nausea and vomiting, liver dysfunction, marrow suppression, mucositis (resulting from high-dose treatment), solar dermatitis	Leukoencephalopathy, osteopenia (resulting from long-term use)
Etoposide, teniposide	Nausea and vomiting, hair loss, mucositis, marrow suppression, allergic reactions (bronchospasm, urticaria, angioedema, hypotension)	Acute myeloid leukemia
Cytarabine	Nausea and vomiting, fever, skin rashes, mucositis, marrow suppression, liver dysfunction, conjunctivitis (from high-dose treatment)	Decreased fertility (with high cumulative dose)
Cyclophosphamide	Nausea and vomiting, hemorrhagic cystitis, marrow suppression, syndrome of inappropriate secretion of antidiuretic hormone, hair loss	Bladder cancer or acute myeloid leukemia (rare), decreased fertility (with high cumulative dose)
Rituximab	Infusion reactions, mucocutaneous reactions, lymphopenia, pulmonary infiltrates	Reactivation of virus infections (hepatitis B), progressive multifocal leukoencephalopathy from JC virus infection
Intrathecal methotrexate	Headache, fever, seizure, marrow suppression, mucositis (in patients with renal dysfunction)	Encephalopathy or myelopathy (with high cumulative dose)
Brain irradiation	Hair loss, postirradiation somnolence syndrome (6–10 weeks after treatment)	Seizure, mineralizing microangiopathy, growth hormone deficiency, thyroid dysfunction, obesity, osteopenia, brain tumors, basal cell carcinoma, parotid gland carcinoma, hair loss, cataract (rare), dental abnormalities

Source: *Williams Hematology*, 9th ed, Chap. 91, Table 91–6.

Prognostic Markers

- Of the many variables that influence prognosis, risk-categorization is the most important.
- Childhood ALL cases are divided into four risk groups: low, standard, high, and very high. Table 54–7 gives details of the three highest categories.
- Adverse prognostic factors in adult cases of ALL cases are shown in Table 54–8.

TABLE 54–7 RISK CLASSIFICATION SYSTEM USED IN ST. JUDE TOTAL THERAPY STUDY XVI

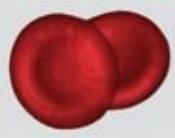
Risk Group	Feature
Standard	Precursor B-cell phenotype in patients ages 1–9 years with a presenting leukocyte count < 50×10^9/L, *ETV6-RUNX1* fusion, or hyperdiploidy (> 50 chromosomes or DNA index > 1.16) Must not have CNS3 status, testicular leukemia, t(9;22), t(1;19), rearranged *MLL* gene, hypodiploidy, or ≥ 0.01% leukemia cells in marrow after 6-week remission induction
High	T-cell acute lymphoblastic leukemia (ALL) and all cases of B-cell precursor ALL that do not meet the criteria for standard or very high risk ALL
Very high	Early T-cell precursor, initial induction failure, or ≥ 1% leukemic cells in marrow after 6-week remission induction

Source: *Williams Hematology*, 9th ed, Chap. 91, Table 91–8.

TABLE 54–8 ADVERSE PROGNOSTIC FACTORS IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

Factors	Precursor B Cell	Precursor T Cell
Age (years)*	> 35	> 35
Leukocyte count ($\times 10^9$/L)	> 30	> 100
Immunophenotype	Pro-B (CD10–)	Pre-T
Genetics	t(9;22) *[BCR-ABL1]* t(4;11) *[MLL-AF4]* Hypodiploidy?	*HOX11L2* expression? *ERG* expression?
Treatment response	Delayed remission (> 4 weeks) Minimal residual disease > 10^{-4} after induction	Delayed remission (> 4 weeks) Minimal residual disease > 10^{-4} after induction

*Continuous factor with increasing age associated with progressively worse outcome.
Source: *Williams Hematology*, 9th ed, Chap. 91, Table 91–7.



For a more detailed discussion, see Richard A. Larson: Acute Lymphoblastic Leukemia, Chap. 91 in *Williams Hematology*, 9th ed.

CHAPTER 55

Chronic Lymphocytic Leukemia and Related Diseases

DEFINITION

- Chronic lymphocytic leukemia (CLL) is a malignancy of mature B cells characterized by blood and marrow lymphocytosis. Varying degrees of lymphadenopathy, splenomegaly, and blood cytopenias develop as the neoplasm progresses.

EPIDEMIOLOGY

- CLL is the most prevalent adult leukemia in Western societies.
- The prevalence of CLL in the United States in 2014 was 126,553 patients. There are approximately 15,700 new cases annually.
- CLL is uncommon before age 40 years and is extremely rare in children or young adults.
- The incidence of the disease increases logarithmically after age 45 years.
- Median age at diagnosis is approximately 70 years.
- CLL is uncommon in Asian countries and in Asians immigrants to the Americas or Europe.

ETIOLOGY AND PATHOGENESIS

Environmental Factors

- Radiation or chemotherapy has not been shown to be a risk factor for developing CLL.
- Exposure to occupational chemicals, such as solvents, paints, or pesticides, has not been established to be a risk factor for CLL.
- There is a higher frequency of living or working on a farm in patients with CLL compared to those without the disease.

Hereditary Factors

- Familial occurrence is most evident in CLL compared with other leukemias.
  - — Multiple cases of CLL are found within a single family with greater frequency.
  - — Up to 10% of patients with CLL have a first- or second-degree relative with CLL.
  - — First-degree relatives of patients with lymphoplasmacytic lymphoma or Waldenström macroglobulinemia (Chap. 69) have a greater than threefold increased risk of developing CLL.
- Genetic factors contribute to increased incidence of CLL.
  - — Polymorphisms in the gene encoding CD5 (located at chromosome 11q13), CD38 (located at chromosome 4p15), or tumor necrosis factor-α, and other genes mapping to chromosome

13q21.33-q22.2.

Disease Biology

- CLL cells typically express CD5, CD19, CD23, and low levels of CD20.
- CLL cells have surface immunoglobulins reactive with self-antigens.
- CLL cells have defective apoptosis supported by the microenvironment.
- CLL cells overexpress BCL-2.
- CLL is characterized by dysregulation in both cellular and humoral immunity.
- CLL cells depend on constitutive activation of the B-cell receptor pathway for survival; the survival signals are transduced through LYN, PI3K, SYK, and BTK pathways.

Monoclonal B-Cell Lymphocytosis

- CLL has an initial phase referred to as monoclonal B-cell lymphocytosis. In most cases, this is not apparent at diagnosis because it is asymptomatic and is detected by flow cell analysis of blood lymphocytes.
- Patients have no lymphadenopathy; no splenomegaly; otherwise normal blood counts; and no fever, night sweats, or unexplained weight loss.
- The incidence of monoclonal B-cell lymphocytosis increases dramatically after 40 years of age.
- Approximately 15% of healthy individuals, who have first-degree relatives with two or more family members with CLL, have blood monoclonal B cells of the CLL B-cell immunophenotype.
- The immunophenotype of the monoclonal B cells may be (1) similar to CLL in approximately 80% of cases (CD5CD23-positive, CD20-dim); (2) atypical CLL (CD5CD23-positive, CD20-bright); or (3) non-CLL type (CD5CD23 negative).
- Monoclonal B-cell lymphocytosis is diagnosed when the number of these cell is less than or equal to 5000×10^9/L. CLL is diagnosed with monoclonal B-cell counts of more than 5000×10^9/L cells.
- Monoclonal B-cell lymphocytosis evolves to CLL at a rate of approximately 1% per year.

CLINICAL FEATURES

- Most patients are asymptomatic at diagnosis. The disease is detected because of a routine blood test showing lymphocytosis or a physical examination confirming lymph node enlargement.
- Some patients have fatigue, reduced exercise tolerance, or malaise.
- Some cases may present with recurrent, usually upper respiratory, infections.
- Patients with advanced disease may have weight loss, recurrent infections, night sweats, fever, and/or symptomatic anemia.
- An unusual finding is insect (mosquito) bite sensitivity with exaggerated cutaneous inflammatory reactions.
- Lymph nodes are generally not fixed or tender.
- The nodes may increase with infections and return to the previous size after resolution.

- Mild to moderate splenomegaly is seen commonly and can cause mild thrombocytopenia.
- Extranodal involvement can include:
  — Hepatomegaly
  — CLL cell infiltrates in the scalp, subconjunctivae, prostate, gonads, or pharynx
  — Pulmonary infiltrates that can be detected on chest imaging
  — Gastrointestinal ulceration, gastrointestinal bleeding, or malabsorption
  — Leukemic cell infiltration of the central nervous system that although unusual, may produce headache, meningitis, cranial nerve palsy, obtundation, or coma

LABORATORY FEATURES

Blood Findings

- CLL is diagnosed with a monoclonal B-cell count of greater than 5000×10^9/L.
- The blood film has an increase in small, normal appearing lymphocytes to a degree related to lymphocyte count; see **Figure 55–1**.
  — Approximately one in five to ten CLL lymphocytes in the blood are ruptured during blood film preparation and are referred to as smudge cells.

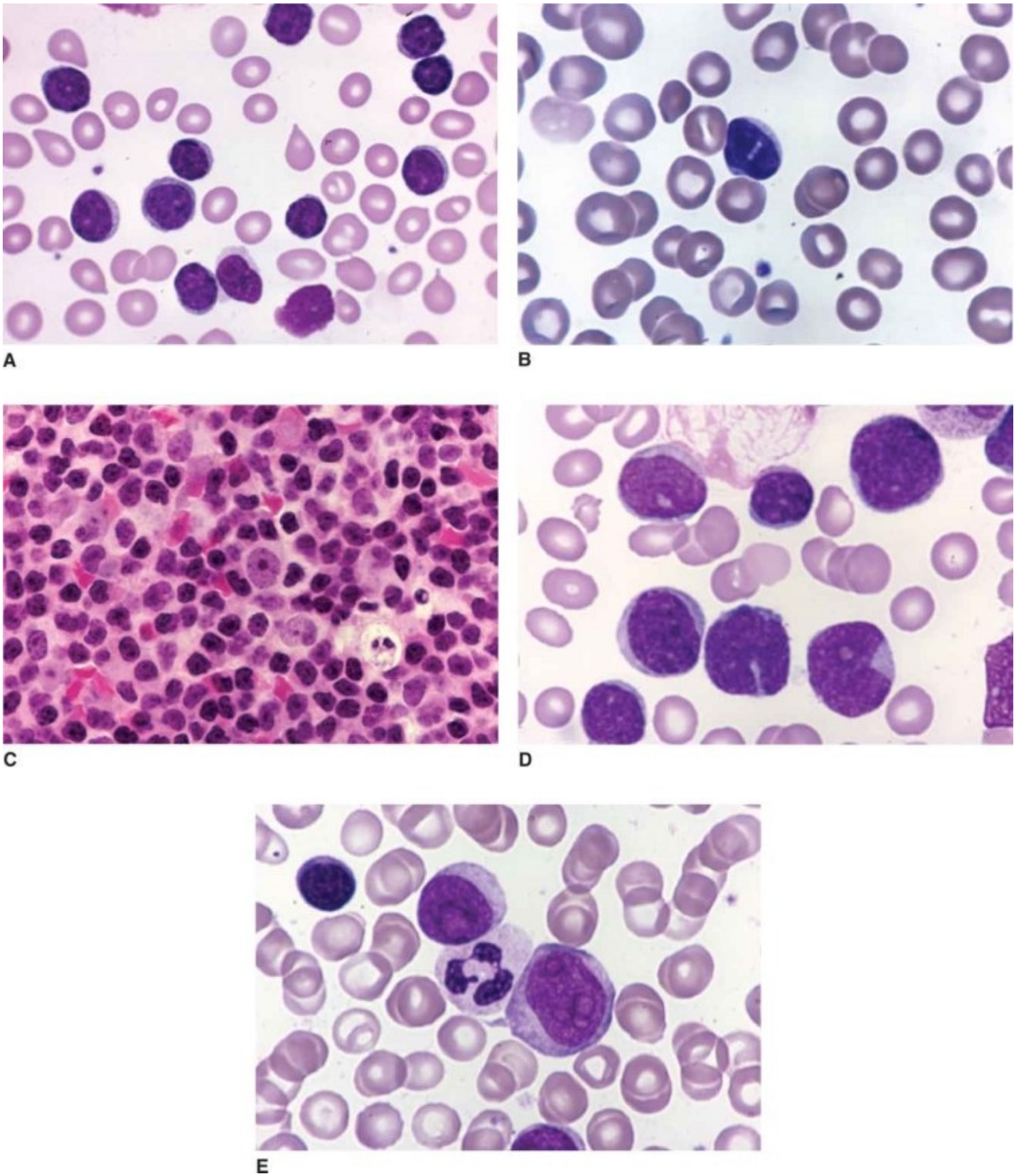


FIGURE 55–1 **A.** Chronic lymphocytic leukemia (CLL). Blood film showing high white cell count composed of small lymphocytes with a high nuclear:cytoplasmic ratio. Smudge cell below. **B.** CLL. Rectangular immunoglobulin crystal in CLL cell. **C.** CLL marrow aspirate film. Marrow replaced by monotonous infiltrate of small lymphocytes (CLL cells). **D.** Large B-cell lymphoma cells in blood. Note occasional nuclear clefts. These cells are similar in appearance of cells in a Richter transformation of CLL. **E.** B-cell prolymphocytic leukemia with larger cells, lower nuclear:cytoplasmic ratio, and easily discernible large nucleoli in cells. Compare to small lymphocyte in upper left, a phenocopy of a characteristic CLL cell, a few of which may be present in prolymphocytic leukemic blood films. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

- Fifteen percent of patients present with normocytic anemia.
- Twenty percent have a positive red cell direct antiglobulin test at some time in the disease.
- Thrombocytopenia caused by antiplatelet antibodies may develop at any time. In advanced disease, thrombocytopenia also may be a result of marrow replacement and/or splenic sequestration.
- Serum lactic dehydrogenase (LDH) and beta-2 microglobulin. Elevation predicts the likelihood of a more aggressive disease.

Marrow Findings

- Marrow is invariably infiltrated with small lymphocytes in one of four patterns:
  — Interstitial (or lacy): 30%; associated with a better prognosis
  — Nodular: 10%
  — Mixed interstitial/nodular: 25%
  — Diffuse marrow replacement: 25%; associated with a poorer prognosis

Lymph Node Findings

- Lymph nodes are affected by diffuse infiltrate of small lymphocytes that efface the node architecture and invade the subcapsular sinus.
- Note that this finding is *not* required for diagnosis.

CLL Cell Immunophenotype

- Diagnosis of CLL requires sustained monoclonal lymphocytosis of greater than 5000×10^9/L cells.
- The diagnosis of CLL requires establishing the monoclonal nature of the lymphocytosis by flow cytometry according to the International Workshop on Chronic Lymphocytic Leukemia (IWCLL) criteria. For example, one usually determines if the light chain expression is either λ or κ (monoclonal) and not both (polyclonal).
- CLL cells typically express CD5CD19,CD23+CD20dim) and CD10CD103–, with low expression of surface immunoglobulin and low-level or absent expression of CD22 and CD79b.
- FMC7, a monoclonal antibody that binds an epitope of CD20 formed when this surface antigen is present at high density, typically does not react with CLL cells.
- CD38 can be detected on the surface of CLL cells using flow cytometry. A level of expression greater than 30% predicts the likelihood of a more aggressive course.
- CD49d expression in more than 30% of the CLL cells predicts the likelihood of a more aggressive course.

Serum Protein and Immunoelectrophoresis

- Most common finding is hypogammaglobulinemia.
- Reduction in the serum levels of IgM precedes that of IgG and IgA.
- Five percent of patients have a serum monoclonal immunoglobulin.
- In some cases, there is defective and/or unbalanced immunoglobulin chain synthesis by the leukemic B-cell clone, resulting in μ heavy-chain disease (see Chap. 70) and/or

immunoglobulin light-chain proteinuria.

Cytogenetic Studies

Chromosome 13 Anomalies

- The most common genetic abnormality found in about half of all patients is deletion of part of the long arm of chromosome 13, specifically at 13q14.-23.1 telomeric to the retinoblastoma gene (*RB-1*) and centromeric to and including the D12S25 region.
- Loss of microRNA *miR15* and/or *miR16–1* might contribute to leukemogenesis and account for the frequent deletions that are observed at 13q14.3.

Chromosome 12 Anomalies

- Trisomy 12 is the second most common abnormality, found in about 20% of patients; half of these have trisomy 12 only.
- This abnormality may not be a primary factor in leukemogenesis but is probably acquired during disease evolution.

Chromosome 11 Anomalies

- Approximately 20% of patients have a deletion(s) in the long arm of chromosome 11, termed 11q-, which is associated with more aggressive disease.
- Associated with younger age at diagnosis (< 55 years) and tend to have bulky cervical lymphadenopathy than patients without such genetic changes.

Chromosome 17 Anomalies

- Deletions in the short arm of chromosome 17 at 17p13.1 are in less than 10% of all patients. The critical gene in the region that typically is deleted is *TP53*.
- Patients with 17p– and/or *TP53* mutations generally have more advanced disease, a higher leukemia-cell proliferative rate, a shorter survival, and greater resistance to first-line therapy.
- These patients are more likely to transform to an aggressive B-cell lymphoma (referred to as a Richter transformation).

Other Chromosome Abnormalities

- Abnormalities involving chromosomes 6, 14, and 18 are less common.

Fluorescence in situ Hybridization

- Using fluorescence in situ hybridization (FISH), the leukemic cells from almost all CLL patients have clonal chromosomal abnormalities.

Immunoglobulin Heavy Chain Gene Mutations

- Two groups of CLL patients are distinguished by the extent that their immunoglobulin heavy chain variable region genes (*IGHV*) have undergone somatic mutation.
  — Approximately 40% of CLL patients have leukemia cells that express nonmutated *IGHV*

genes and have a greater tendency for disease progression.
  — Approximately 60% express *IGHV* mutated genes with levels of base substitutions that distinguish them from their germ-line counterparts. Patients with mutated *IGHV* have a significantly longer treatment-free interval, longer remission duration if therapy is required, and longer survival after diagnosis.
- One noted exception to this distinction is represented by patients who have leukemia cells that use a particular immunoglobulin gene, designated *IGHV3–21*.
  — Patients who have CLL cells that use a mutated *IGHV3–21* gene together with a λ immunoglobulin light chain encoded *IGHV3–21* have a risk of aggressive disease similar to that of patients who have leukemia cells that express unmutated IgHv genes.

EVALUATION

- The diagnosis is by detection of a monoclonal B-cell lymphocytosis of at least 5000 cells × 10^9/L.
- A lymphocyte doubling time of less than 12 months has been shown to be associated with a more aggressive course.
- Flow cytometry of blood cells should show the cells to be monoclonal CD5CD19CD23-positive, CD20-dim, express dim surface immunoglobulin, and be CD10CD79b-negative.
- Neither a marrow aspirate and biopsy nor lymph node biopsy is required for the diagnosis in the overwhelming majority of patients.
- Computed tomography (CT) or other imaging is not necessary for the initial evaluation of patients with CLL. Positron emission tomography (PET) can be useful if a Richter transformation is suspected.
- FISH cytogenetic studies should be done to identify the abnormalities commonly seen in patients with CLL (**Table 55–1**).

TABLE 55–1 **SURVIVAL OUTCOMES AND TIME TO FIRST TREATMENT BASED ON FISH CYTOGENETICS AND IGHV STATUS**

	Prognostic Variables	Median Survival (Months)	Median Time to First Treatment
Interphase FISH cytogenetics	13q– (sole)	133	92 months
	Trisomy 12	114	33 months
	Normal	111	49 months
	11q–	79	13 months
	17p–	32	9 months
IGHV mutational status	Unmutated (≥ 98%)	89	3.5 years
	Mutated (< 98%)	>152	9.2 years

FISH, fluorescence in situ hybridization; IGHV, immunoglobulin heavy-chain variable region.
Source: *Williams Hematology*, 9th ed, Chap. 92, Table 92–1.

- Patients with anemia without reticulocytosis should be evaluated for their anemia including serum vitamin B_{12}, red cell folic acid levels, serum ferritin, and tests for gastrointestinal

bleeding, as appropriate.

- Patients with CLL frequently have hypogammaglobulinemia and occasionally have a monoclonal Ig; serum electrophoresis and measures of serum IgG, IgM, and IgA are important.
- The assessment of IgHv somatic mutation by a polymerase chain reaction (PCR) assay is an important prognostic tool. Patients with less than 2% homology in their nucleotide sequence compared to consensus germline sequence are considered unmutated (ie, about 40% of patients) and have a poorer prognosis.
- **Table 55–2** summarizes frequently used prognostic indicators.

TABLE 55–2 OUTCOMES OF SELECTED PROGNOSTIC FACTORS

	Favorable Outcome	Unfavorable Outcome
Lactate dehydrogenase	Low or normal	Elevated
Lymphocyte doubling time	> 12 months	≤ 12 months
Thymidine kinase activity	Low or normal	Elevated
β2-microglobulin	Low or normal	Elevated
Soluble CD23 levels	Low or normal	Elevated
CD38 expression	< 30%	> 30%
Interphase FISH cytogenetics	Normal	11q–
	Trisomy 12	17p–
	13q– (sole)	
IGHV mutational status	Mutated	Unmutated
CD49d expression	< 30%	> 30%

FISH, fluorescence in situ hybridization; IGHV, immunoglobulin heavy-chain variable region.
Source: *Williams Hematology*, 9th ed, Chap. 92, Table 92–2.

Determining the Clinical Stage of CLL

- One of two staging systems is usually used: the Rai system defined in **Table 55–3** or the Binet system characterized in **Table 55–4**.

TABLE 55–3 RAI CLINICAL STAGING SYSTEM

Stage at Diagnosis	Risk Level	Rai Stage at Diagnosis	Patients Never Requiring Therapy (%)	Median Survival (Months)
0	Low risk	Lymphocytosis > 5 $\times 10^9$/L only	59	150
1	Intermediate	Lymphocytosis and lymph nodes (LN) enlargement	21	101
2		Lymphocytosis and spleen/liver (S/L) enlargement ± LN	23	71
3	High	Lymphocytosis and anemia (with hemoglobin < 11 g/dL) ± LN or S/L	5	19
4		Lymphocytosis and thrombocytopenia (< 100 $\times 10^{12}$/L ± LN) or S/L	0	19

Source: *Williams Hematology*, 9th ed, Chap. 92, Table 92–3.

TABLE 55–4 BINET CLINICAL STAGING SYSTEM

Stage at Diagnosis	Equivalent Rai Staging	Clinical Features at Diagnosis	Proportion of Patients (%)	Median Survival, Years
A	0-2	Lymphocytosis > 5×10^9/L only with < 3 enlarged nodal areas*; no anemia, no thrombocytopenia	15	12+
B	1-2	Lymphocytosis > 5×10^9/L + ≥ 3 enlarged nodal areas*; no anemia, no thrombocytopenia	30	7
C	3-4	Lymphocytosis > 5×10^9/L + anemia (hemoglobin < 10 g/dL) or thrombocytopenia (< 100×10^{12}/L) regardless of the number of enlarged nodal areas*	55	2

*Nodal areas counted as one each of the following: axillary, cervical, inguinal lymph nodes, whether unilateral or bilateral, spleen, and liver.
Source: *Williams Hematology,* 9th ed, Chap. 92, Table 92–4.

- Both these systems consider the degree and distribution of lymphadenopathy and the presence of anemia and or thrombocytopenia, but the Rai staging classification considers hepatic and splenic enlargement, whereas the Binet staging system does not.

DIFFERENTIAL DIAGNOSIS

- See **Table 55–5** for the immunophenotype of chronic B-cell leukemias/lymphomas.

TABLE 55–5 IMMUNOPHENOTYPE OF CHRONIC B-CELL LEUKEMIAS/LYMPHOMAS

Disease Entity	sIg	CD5	CD10	CD11C	CD19	CD20	CD22	CD23	CD25	CD103
Chronic lymphocytic leukemia	+/–	++	–	–/+	+	+/–	–/+	+ +	–/+	–
Prolymphocytic leukemia	++	+	–	–/+	+	+/–	+	+/–	–	–
Hairy cell leukemia	+/–	–/+	–	++	+	+	++	–/+	+	++
Mantle cell lymphoma	+	++	–	–	+	+	+	–/+	–	–
Splenic marginal zone lymphoma	+	–/+	–/+	+	+	+	+/–	–/+	–	–
Lymphoplasmacytoid lymphoma	+/–	–/+	–	–	+	+/–	+/–	–/+	+/–	–
Follicular center lymphoma	+	–	+	–	+	++	+	–/+	–	–

sIg, surface immunoglobulin.
– Leukemia cells do not express the surface antigen; + leukemia cells from most cases express the surface antigen; +/–, low-level expression; –/+, most cases either do not express the antigen or express it at very low levels; ++, high-level expression of the surface antigen in nearly all cases.
Source: *Williams Hematology,* 8th ed, Chap. 94, Table 94–1.

- Monoclonal lymphocytosis versus causes of polyclonal lymphocytosis (see Chap. 49)
- Prolymphocytic leukemia (see discussion later in this chapter)
  - — More than 50% of circulating leukemic lymphocytes have prolymphocytic morphology, with a larger size than CLL cells and a prominent nucleolus.
  - — There are high levels of CD79b and surface immunoglobulin and low levels of CD5.

- Hairy cell leukemia (see Chap. 56)
- Lymphomas with circulating neoplastic cells (see Chap. 60)
- Small cell lymphocytic lymphoma
  — Low-grade small lymphocytic B-cell lymphoma is closely related to B-cell CLL in its biology and clinical features.
  — There is lymph node involvement.
  — There is no overt blood or marrow involvement.
- Mantle cell lymphoma (see Chap. 62)
  — It expresses many of the same surface antigens as CLL B cells.
  — It does not express CD23.
  — The t(11;14) is characteristic of mantle cell lymphoma.
- Splenic marginal zone lymphoma (SMZL) (see Chap. 63)
  — This is commonly called splenic lymphoma with villous lymphocytes.
  — SMZL has a mature B-cell phenotype and expresses IgM and IgD but typically lacks expression of CD23, CD43, CD10, BCL-6, and cyclin D.
  — Neoplastic B cells have weak or absent expression of CD5
- Lymphomas of follicular center cell origin (see Chap. 61)
  — Small cleaved cell lymphomas express the CD10 (CALLA) antigen.
- Lymphoplasmacytic leukemias
  — These express CD38, PCA-1, CD56, and CD85, but there is low-level or lack of expression of CD19, CD20, CD24, CD72, and HLA-DR.
- Waldenström macroglobulinemia (see Chap. 69)
- Myeloma (see Chap. 68)
- T-cell lymphoproliferative disorders (see Chap. 66)
- T-cell CLL and T-cell prolymphocytic leukemia (see discussion later in this chapter)
- Large granular lymphocytic leukemia (see Chap. 57)
- Adult T-cell leukemia/lymphoma (see Chap. 66)
- Cutaneous T-cell lymphomas (see Chap. 65)

TREATMENT

General Considerations

- Not all patients require treatment at time of diagnosis.
- No evidence of a survival advantage apparently occurs if therapy is initiated when the patient is asymptomatic.
- Presence of del(17p) predicts resistance to standard chemotherapy and thus an inferior prognosis.
- Presence of del(11q) has significantly lower response rates to single-agent chemotherapy; outcomes are improved with fludarabine-based combination therapy.

Indications for Treatment

- Treatment is indicated for patients with active disease, including those with:
  — Constitutional symptoms, such as weakness, painful lymphadenopathy, fever, night sweats,

and weight loss
— An increase of 50% or more in blood lymphocyte count over a 2-month period or lymphocyte count doubling time of less than 6 months
— Rapidly enlarging lymph nodes, spleen, or liver.
— Autoimmune complications (eg, autoimmune hemolytic anemia or autoimmune thrombocytopenia). Poor response to therapy (typically glucocorticoids) directed at the autoimmunity is an indication for treatment of the underlying CLL.
— Repeated episodes of infection. Patients with hypogammaglobulinemia with infection should receive periodic infusions of gamma globulin.
— Transformation of CLL cells to a more aggressive histology causing worsening anemia and/or thrombocytopenia (prolymphocytic or Richter transformation)

Approach to Therapy

Localized Disease

- Irradiation remains a useful technique for localized treatment.
- Delivery of 200 Gy often can result in rapid shrinkage of lymph nodes or masses.

Initial Treatment of Active Disease

- Goals of therapy are to ameliorate symptoms and improve survival.
- No therapy, other than possibly allogeneic hematopoietic stem cell transplantation, has been shown to cure CLL.
- Several initial treatment options exist; most have not been directly compared. Enrollment in a clinical trial is recommended.
- Initial therapy choice should be based on goals of treatment as well as patient (age, comorbidities) and high-risk disease characteristics such as del(17p) or del(11p).
- Regimens differ in rates of complete remission, time to progression, and toxicities.
  — Single agent chemotherapy with drugs such chlorambucil, cyclophosphamide, bendamustine, and fludarabine can be utilized.
  — Combination chemotherapy produces a higher response rate and higher complete remission rate but at the risk of more toxicity.

Fludarabine-based therapy (Table 55–6)

- Combination therapy with fludarabine and rituximab (FR) is often used.
- There is a higher response rate and higher complete remission rate but more toxicity with a combination of fludarabine, cyclophosphamide, and rituximab (FCR). This regimen is often considered a standard of care for physically fit patients but should be used cautiously in older patients.
- Prognosis of patients with del(11q) is improved with FCR therapy; however, that of del(17p) remains poor.

TABLE 55–6 **FLUDARABINE-BASED CHEMOTHERAPY FOR CHRONIC LYMPHOCYTIC LEUKEMIA**

	Dose/Route of

Regimen	Cycle Length	Administration	When Given
Fludarabine and rituximab (FR)	28 days		
Fludarabine		25 mg/m^2 IV	All cycles: days 1–5
Rituximab		375 mg/m^2 IV	Cycle 1: days 1 and 4 Cycles 2–6: day 1
Fludarabine, cyclophosphamide, and rituximab (FCR)	28 days		
Fludarabine		25 mg/m^2 IV	Cycle 1: days 2–4 Cycles 2–6: days 1–3
Cyclophosphamide		250 mg/m^2 IV	Cycle 1: days 2–4 Cycles 2–6: days 1–3
Rituximab		Cycle 1: 375 mg/m^2 IV Cycles 2–6: 375–500 mg/m^2 IV	All cycles: day 1

IV, intravenously.

Bendamustine-based Therapy

- Bendamustine with rituximab (BR) has been tested in both patients with untreated CLL and relapsed disease.
- In the phase III, multicenter, CLL10 trial of 564 patients with previously untreated CLL, good performance status and low comorbidity burden randomized to FCR or BR, the overall response rates were similar, but complete response rates were higher in the FCR arm (40% FCR, 31% BR). FCR was more toxic.

Pentostatin-based Therapy

- Combination therapy results in higher response rates than with single agent.
- Pentostatin can be used instead of fludarabine, but the relative effectiveness of the most common combination (pentostatin, cyclophosphamide, and rituximab) is not established.

Chlorambucil-based Therapy

- This agent is often used as single agent in older patients who are not candidates for combination therapy.
- Combination of chlorambucil with an anti-CD 20 monoclonal antibody (eg, rituximab, obinutuzumab, ofatumumab) results in higher response rates and longer progression-free survival but has greater toxicity.

Anti-CD20 Monoclonal Antibodies

- Rituximab, ofatumumab, and obinutuzumab are all effective in CLL.
  — Rituximab as a single-agent resulted in only partial responses in a minority of CLL patients. Rituximab has been most widely used and is more effective in combination therapy.
  — Ofatumumab is approved by the US Food and Drug Administration (FDA) for use (1) in combination with chlorambucil for the treatment of patients with previously untreated CLL who are not candidates for fludarabine-based treatment and (2) as a single agent for

patients with relapsed or refractory disease.
— Obinutuzumab is approved by the FDA for use in combination with chlorambucil for patients with previously untreated CLL. It is significantly more active than chlorambucil plus rituximab).

Ibrutinib

- This Bruton tyrosine kinase (BTK) inhibitor with a high response rate in patients with relapsed CLL and a high level of activity as initial therapy in patients with deletion 17p. It is FDA approved for these two indications.
  — Even in patients with relapsed disease, the responses are often durable as long as the patient continues to tolerate the drug.
  — The most frequent side effects are diarrhea, nausea, fatigue, and a higher incidence of atrial fibrillation.
  — Some patients will have a bleeding diathesis that makes combining ibrutinib with anticoagulants—particularly warfarin—problematic.
  — Patients starting on ibrutinib will typically develop lymphocytosis that will resolve spontaneously over time.

Therapy of Relapsed/Refractory CLL

- Patients with asymptomatic recurrent CLL may not require therapy but should be followed closely for developing of active disease, which requires therapy.
- Choice of therapy at relapse should be based on quality and duration of response to prior therapy.
  — Patients who relapse more than 1 year after an initial response may be retreated with same therapy as for initial treatment. Ibrutinib or rituximab plus idelalisib are appropriate alternatives.
  — The combination of rituximab and idelalisib, an oral inhibitor of phosphoinositide 3′-kinase (PI3K) resulted in an overall response rate of 81% versus rituximab and placebo. Idelasib plus rituximab was FDA approved in 2014 for treatment of patients with relapsed CLL with significant comorbidities.
  — Patients with early relapsed CLL or whose disease is refractory to initial therapy should be treated with a second-line regimen; young patients with good performance status and few comorbidities may be considered for hematopoietic stem cell transplantation on a clinical trial. The combination of rituximab and idelalisib, an oral inhibitor of phosphoinositide 3′-kinase (PI3K) resulted in an overall response rate of 81% versus rituximab and placebo. Idelasib plus rituximab was FDA approved in 2014 for treatment of patients with relapsed CLL with significant comorbidities.
- Palliation of symptoms may be achieved with rituximab, ofatumumab, and glucocorticoids with only moderate toxicity. Splenectomy or splenic irradiation may be useful for patients with splenomegaly and severe cytopenias refractory to systemic therapy. Localized irradiation may improve symptoms related to bulky disease or impingement on vital organs.

Hematopoietic Stem Cell Transplantation

- Due to potential morbidity and mortality associated with hematopoietic stem cell transplantation (HSCT), only select patients are considered and, if possible, HSCT should be performed in context of a clinical trial.
- Ideal timing is unknown. May be considered for patients with clinically aggressive relapsed/refractory CLL or those with high risk genetic factors, including del(17p) and del (11q).

Autologous HSCT

- Preparative regimen is well tolerated.
- Risk of recurrent disease after transplant is high.
- This type of HSCT may prolong disease-free survival.

Allogeneic HSCT

- This procedure may eradicate the leukemia cells to the levels that cannot be detected using sensitive molecular techniques to detect clonal immunoglobulin gene rearrangements.
- Patients who relapse following allogeneic HSCT may respond to infusions of donor leukocytes.

Nonmyeloablative Allogeneic HSCT

- Preparative regimen is better tolerated than standard allogeneic HSCT.
- Complete chimerism, as well as best response, is not achieved immediately after transplantation but may take over 3 months to develop.
- Patients with refractory CLL can experience eradication of minimal residual disease (MRD) several weeks following transplant, providing evidence of a graft-versus-leukemia effect.

Response Criteria

- Response criteria for patients who have had prior therapy are the same as those used for initial therapy.

Complete Response

- This requires that the patient is free of clinical disease for at least 2 months after therapy.
- Satisfactory blood counts:
  — Hemoglobin > 11 g/dL; no red cell transfusion requirement
  — Neutrophil count at least 1.5×10^9/L
  — Lymphocyte count $\leq 4.0 \times 10^9$/L
  — Platelets $\geq 100 \times 10^9$/L
- There is an absence of fever, night sweats, weight loss, or other disease-related symptoms.
- There is an absence of hepatosplenomegaly or detectable adenopathy.
- Marrow has less than 30% lymphocytes and lacks pathologic lymphoid nodules.
- If the marrow is found to be hypocellular, a repeat marrow biopsy should be performed after 4 to 6 weeks, provided blood counts have recovered. The marrow biopsy should not exceed a period of 6 months after the last treatment.

Partial Response

- For at least 2 months after therapy, the patient must have at least:
  — A 50% reduction in the number of blood lymphocytes
  — A 50% reduction in lymphadenopathy or hepatosplenomegaly
- Absolute neutrophil count of at least 1.5×10^9/L or greater than 50% improvement over that noted prior to therapy
- Blood platelets $>100 \times 10^9$/L
- Hemoglobin >11 g/dL
- A 50% improvement in platelet or red cell counts over pretreatment values without transfusion
- Patients who satisfy the criteria for a complete response but who have persistent lymphoid nodules in the marrow are classified as having a *nodular partial response*.

Progressive Disease

- New lymphadenopathy; an increase in lymphadenopathy of greater than or equal to 50%
- An increase in the liver or spleen size of greater than 50% or the appearance of hepatomegaly or splenomegaly while on therapy
- An increase in the absolute lymphocyte count of greater than or equal to 50%
- Transformation to a more aggressive histology (eg, Richter syndrome), which should be established by lymph node biopsy

Refractory Disease

- Patient who experiences disease progression within 6 months of completing therapy is considered to have disease that is refractory to such therapy.

Minimal Residual Disease

- Improved leukemia-cell detection methods using flow cytometry or PCR can reveal patients in complete response who have residual CLL cells.
- Patients who experience eradication of minimal residual disease (MRD) apparently have a longer treatment-free survival than do patients who have achieved a complete response but have persistent MRD.

COURSE AND PROGNOSIS

Disease Complications

Infection

- Infection is a major cause of morbidity or mortality.
- Susceptibility to infection correlates with hypogammaglobulinemia and/or T-cell lymphocytopenia.
  — Advanced-stage disease, hypogammaglobulinemia, and low levels of specific antibodies to pneumococcal capsular polysaccharide are associated with severe or multiple infections.
- Monthly intravenous administration of pooled normal serum immunoglobulin (intravenous immune globulin [IVIG] at 240 to 400 mg/kg every 3 to 4 weeks) may decrease frequency of

infection.

Autoimmune Complications

- These conditions include autoimmune hemolytic anemia, autoimmune thrombocytopenia, and pure red cell aplasia.
- In the majority of patients, the nonmalignant B-cell clone produces the autoantibody.
- Glucocorticoids have been the mainstay of treatment for autoimmune hemolytic anemia and autoimmune thrombocytopenia. Many patients relapse after discontinuation and may need therapy with IVIG or rituximab.
- Pure red cell aplasia is rare; treatment with glucocorticoids, cyclophosphamide, and/or cyclosporine result in slow correction of anemia over several weeks.

Second Malignancies

- Most frequent are melanoma, sarcoma, colorectal or lung cancers, or myeloma.
- Higher recurrence rates of basal cell carcinoma occur in individuals after Mohs' surgery (a surgical procedure used to treat common types of skin cancer) than in the general population.
- The incidence of developing Merkel cell carcinoma (rare and highly aggressive cancer in which malignant cancer cells develop on or just beneath the skin and in hair follicle is greater.
- The risk of developing more aggressive and/or metastatic squamous cell skin carcinomas is greater than in the general population.
- Multiple myeloma occurs at 10 times the expected rate in patients with CLL but evidently does not arise from the same malignant B-cell clone.
- Both untreated and treated CLL patients can develop acute myelogenous leukemia or myelodysplastic syndrome.
- Therapy-related acute myelogenous leukemia may develop after treatment with single-agent deoxyadenosine analogs, such as fludarabine or cladribine.

Richter Transformation

- This condition represents transformation to an aggressive, high-grade, large B-cell lymphoma.
- It can occur at any time in the course of CLL.
- It occurs in approximately 3% of patients at a median of 2 years after diagnosis of CLL.
- It can develop in patients who had not received chemotherapy.
- It arises from the original CLL clone.
- Chromosomal abnormalities are complex and include:
  — del 8p, del 9p, del 11q (11q23), 12(+), del 13q, 14q(+), del 17p, del 20, and/or translocations involving chromosome 12
  — Trisomy 12 and chromosome 11 abnormalities (which are more frequent)
- There is a higher incidence of *P53* mutations at transformation.
- Three independent risk factors for transformation are identified.
  — High-level expression of CD38 by leukemia B cells
  — Absence of leukemia-cell deletion at 13q14
  — Leukemia cell expression of certain *IgHV* genes, notably *IGHV4-39*
- Clinical and laboratory features include the following:

— Increased serum LDH activity in approximately 80% of patients
— Rapid lymph node enlargement in approximately 65%
— Fever and/or weight loss in approximately 60%
— Monoclonal gammopathy in approximately 45%
— Extranodal disease in approximately 40%

- Not all patients with CLL that have rapid lymph node enlargement have Richter transformation.
- Infection with herpes simplex virus can cause acute lymphadenitis.
- Occasional cases of Richter transformation have histology resembling that of Hodgkin lymphoma (see Chap. 59). This is termed *Richter syndrome with Hodgkin lymphoma features*.
  — Richter syndrome with Hodgkin lymphoma features may respond favorably to therapy for Hodgkin lymphoma.
- Treatment similar to that of patients with high-grade lymphoma (see Chap. 60).
- Encouraging responses have been observed with OFAR regimen (oxaliplatin, fludarabine, cytarabine, rituximab).
- Median survival is 5 months after transformation.

CLL/Prolymphocyte and Prolymphocytic Transformation

- Fifteen percent of patients with CLL have a mixture of small lymphocytes and prolymphocytes (PL), the latter accounting for 10% to 50% of the lymphoid cells. These patients are considered to have CLL/PL.
- In 80% of CLL/PL cases, the proportion of PLs remains stable.
- Twenty percent of patients with CLL/PL undergo prolymphocytic transformation with greater than 55% of the leukemia cells having PL morphology (see below).
  — Progressive splenomegaly is characteristic.
  — Patients have a mean survival of 9 months after transformation.

Acute Lymphoblastic Leukemia

- Rare complication
- Can arise from same cell clone as CLL
- Associated with high levels of expression of *c-MYC* and surface immunoglobulin

Prognosis for CLL

- No established cures for CLL exist.
- Spontaneous remissions are rare.
- CLL varies substantially between different patients depending on clinical stage and/or presence or absence of certain disease features associated with progression and/or adverse clinical outcomes.
- Female patients have longer survival.
- Male-to-female ratio is greater in patients diagnosed at younger ages (< 65 years).
- CLL relegated deaths occur more frequently in younger patients.
- Five-year relative survival is approximately 70%, 70%, 65%, and 40% for age groups younger than 40, 40 to 59, 60 to 79, and 80+ years, respectively, indicating that the 5-year

survival does not vary significantly between the different age groups under the age of 80 years.
- Risk of CLL-unrelated deaths and secondary malignancies predominate in older age groups.

B-CELL PROLYMPHOCYTIC LEUKEMIA (PLL)

Definition

- PLL is a clinical and morphologic variant of CLL.
- It is a subacute lymphoid leukemia.
- Incidence is less than 10% that of CLL.
- Diagnosis of PLL requires that at least 55% of the circulating leukemic lymphocytes have prolymphocytic morphology.

Etiology and Pathogenesis

- PLL is a de novo leukemia as are most chronic and subacute lymphocytic leukemias.

Cytogenetics

- Karyotype of the leukemia cells from many patients displays the 14q+ abnormality.
- Trisomy 12 is another recurrent abnormality.
- Deletions of the long arm of chromosome 6 (6q-) and rearrangement affecting chromosomes 1 and 12 are occasionally observed.
- The most common abnormalities are as follows:
  — 13q14 at 46%
  — Trisomy 12 at 21%
  — 14q32 at 21%
- Loss of heterozygosity at 17p13.3 associated with inactivating mutations in the *TP53* gene is observed in as many as three-fourths of the cases examined.

Cytogenesis

- Mature B-cell origin that have undergone immunoglobulin gene rearrangement.
  — IGHV4-34 genes.

Clinical Features

- Fifty percent of patients are older than 70 years.
- Patients often have advanced disease at presentation.
- Presenting symptoms include fatigue, weakness, weight loss, an acquired bleeding tendency, and early satiety because of splenomegaly.
- Massive splenomegaly occurs in about two-thirds of patients.
- Patients typically have relatively little palpable lymphadenopathy.
- In rare cases, patients may present with leukemic meningitis, leukemic pleural effusion, or malignant ascites.

Therapy, Course, and Prognosis

- Indications for treatment include the following:
  — Disease-related symptoms
  — Symptomatic splenomegaly
  — Progressive marrow failure
  — Blood prolymphocyte count > 20×10^9/L
  — Hemoglobin < 10 g/dL in the absence of hemolysis
  — Platelet count < 100×10^9/L
- Treatment is with alkylating agents similar to those used for CLL, or combinations such as CHOP, yield response rates of 20%.
- Fludarabine or cladribine may be effective.
- Monoclonal antibodies (eg, alemtuzumab, rituximab), alone or in combination with chemotherapy, may be effective.
- Splenic irradiation with 1000 to 1600 Gy delivered to the splenic bed has been advocated as a palliative therapy for the disease.

T-CELL PROLYMPHOCYTIC LEUKEMIA (PLL)

Definition

- Because of its relative aggressive clinical behavior, what formerly had been called T-cell CLL is categorized in the REAL classification as T-cell PLL regardless of leukemia cell morphology (see Chap. 54).
- Incidence is less than 5% that of CLL.

Etiology and Pathogenesis

- Etiology is unknown.
- There is a 3:2 male-to-female predominance.
- Incidence is five to six times higher in the southern islands of Japan than in Western societies.
- Infection with human T-lymphotropic virus type I (HTLV-1) may play a pathogenic role in a subset of patients.

Genetics

- Chromosomal regions most often overrepresented are:
  — 8q at 75%, 5p at 62%, and 14q at 37%, as well as 6p and 21 both at 25%
- Chromosomal regions most often underrepresented are:
  — 8p and 11q at 75%, 13q at 37%, and 6q, 7q, 16q, 17p, and 17q at 25%
- Less common cytogenetic rearrangements are:
  — del(6)t(X;6), (p14;q25), del(13)t(13;14)(q22;q11), t(5;13)(q34;p11), r(17)(p13q21), and t(17;20)(q21;q13)
- Alterations on chromosomes 5, 6, 8, 11, 13, 14, 17, and/or 21 apparently cluster into discrete regions that may contain genes that are deleted or amplified during leukemogenesis or disease progression.
- Strong association with mutations in the ataxia-telangiectasia–mutated gene that maps to chromosome region 11q22.3–23.1.

- *MCP1* or *TCL1* on the short arms of chromosome 13 and 14, respectively, are implicated in the pathogenesis of T-PLL.

Clinical Features

- Presenting symptoms include fatigue, weakness, weight loss, and early satiety because of splenomegaly.
- About a third of patients have cutaneous involvement on the torso, arms, and face, which is usually present at diagnosis.
- Skin manifestations include diffuse infiltrated erythema and erythroderma, producing a nonscaling, papular, nonpruritic rash.
- Some cases present with central nervous system involvement.

Laboratory Features

- Blood lymphocyte counts often in excess of 10×10^9/L at presentation.
- T-lymphocyte infiltration of the marrow is present.
- Biopsy of erythematous skin lesions can reveal a perivascular or periappendiceal dermal infiltrate of lymphoid cells, often with prolymphocyte morphology.
- Leukemia cells typically express pan-T-cell differentiation antigens (eg, CD2, CD3, CD5, and CD7), but not CD1, HLA-DR, or terminal transferase, reflecting a mature T-cell phenotype.
- In addition to pan-T-cell surface antigens, approximately:
  — Seventy-five percent of cases express CD4 (helper T-cell phenotype), but not CD8.
- Fifteen percent of cases express CD8 (suppressor/cytotoxic T-cell phenotype) but not CD4.
- Ten percent of cases express both CD4 and CD8 (less-mature T-cell phenotype).
- Leukemia cells have monoclonal T-cell receptor gene rearrangements.

Differential Diagnosis

Polyclonal T-Cell Lymphocytosis (Chap. 49)

- Cells typically are a mixture of $CD4^+/CD8^-$ and $CD4^-/CD8^+$ T cells and lack monoclonal T-cell receptor gene rearrangements.

T-Cell Large-Granular Lymphocytic Leukemia (see Chap. 57)

- Leukemia cells have large granular lymphocyte morphology.

Adult T-Cell Leukemia/Lymphoma (see Chap. 66)

- This condition is endemic to the southwest of Japan and the Caribbean region.
- Patients have lymphadenopathy, hypercalcemia, and high white blood cell counts.
- Leukemia cells have polylobed or convoluted nuclei.
- Patients typically have antibodies to HTLV-I.

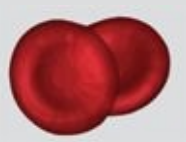
Mycosis Fungoides and Sézary Syndrome (see Chap. 65)

- Neoplastic cells have characteristic cerebriform nuclei.

- This condition shares many features with T-cell PLL.

Therapy, Course, and Prognosis

- This aggressive disease is generally refractory to conventional alkylating agent chemotherapy.
- Treatment with deoxyadenosine analogues is effective in inducing complete response or partial response in about half of patients.
- Topical glucocorticoids, mechlorethamine, carmustine, ultraviolet light B, PUVA, or total skin electron beam therapy is palliative for patients with extensive cutaneous involvement (see Chap. 65).
- Clinical trials have found that alemtuzumab induced responses in more than two-thirds of heavily pretreated relapsed/refractory patients with T-cell PLL.
- Systemic glucocorticoids may be palliative.
- High-dose chemoradiotherapy and allogeneic stem cell transplantation have had anecdotal success.



For a more detailed discussion, see Farrukh T. Awan and John C. Byrd: Chronic Lymphocytic Leukemia and Related Diseases, Chap. 92 in *Williams Hematology*, 9th ed.

CHAPTER 56

Hairy Cell Leukemia

DEFINITION

- This B-lymphocyte malignancy principally involves the marrow and spleen.
- Blood cytopenias and marrow reticulin fibrosis are frequent features.
- Irregular cytoplasmic projections on neoplastic B lymphocytes (which gives the disease its name) are most striking when examined as a wet preparation by phase microscopy.

EPIDEMIOLOGY

- It is estimated that about 700 cases per year occur in the United States (approximately 2% of all leukemias).
- The male-to-female ratio is approximately 4:1.
- The median age at presentation is approximately 55 years.
- There is a bimodal peak incidence by age with a mode at approximately age 30 and at age 55.
- More than 90% of patients are of European descent.
- Disease is rare in persons of African or Asian descent.

ETIOLOGY AND PATHOGENESIS

- No exogenous causes have been established.
- A mutation in *BRAF* (ie, $BRAF^{V600E}$) is found in virtually all cases. This mutation is not found in cases of variant hairy cell leukemia.
- Hairy cells are B cells in a late (preplasma cell) stage of development.
- Neoplastic B cells have clonal immunoglobulin gene rearrangements.
- Neoplastic B cells express pan-B–cell markers (eg, CD19, CD20, and CD22) and the plasma cell marker prostate cancer antigen-1.
- Neoplastic B cells express additional surface antigens that are uncommon on B lymphocytes (eg, CD11c, CD25, and CD103).
- Neoplastic B cells secrete cytokines that may impair normal hematopoiesis (eg, tumor necrosis factor-α).

CLINICAL FEATURES

- Abdominal fullness/discomfort is caused by massive splenomegaly (25%).
- Fatigue, weakness, and weight loss may occur (25%).

- Bleeding or infection may be present (25%).
- Some patients are found incidentally to have abnormal blood count and/or splenomegaly (25%).
- Painful bony lesions rarely occur (3%).
- Splenomegaly exists in 90% of patients (median splenic weight approximately 1300 g).
- Infections with common bacteria, viruses, fungi, *Mycobacterium kansasii*, *Pneumocystis jiroveci*, aspergillus, histoplasma, cryptococcus, *Toxoplasma gondii* or other opportunistic organisms, once common, are less frequent because of more effective initial therapy.
- Unusual findings include cutaneous vasculitis, leukoclastic angiitis, erythema nodosum, polyarthritis, and Raynaud phenomenon.

LABORATORY FEATURES

- Anemia is present in three-fourths of patients.
- Eighty percent of patients have absolute neutropenia and monocytopenia.
- Severe neutropenia ($< 0.5 \times 10^9$/L) is found in 30% of patients.
- Severe monocytopenia is hallmark of the disease.
- Thrombocytopenia occurs in about 75% of patients.
- Moderate to severe pancytopenia is found in approximately 70% of patients.
- Careful examination of the blood by light microscopy identifies hairy cells in 80% of patients (**Figure 56–1**).
- Liver function test abnormalities occur in 19% of cases, azotemia in 27%, and hyperglobulinemia in 18%, which may be monoclonal.
- Occasionally, leukocytosis is present as a result of circulating hairy cells. Extreme leukocytosis ($> 100 \times 10^9$/L) can occur very infrequently, most often seen in the "hairy cell leukemia variant" (see below).
- Hairy cells comprise less than 20% of lymphocytes in patients with low white blood cell counts but are the predominant cell in patients whose white blood cell count is greater than 10×10^9/L.
- Marrow biopsy shows focal or diffuse infiltrate of leukemic cells with characteristic surrounding halo of pale-staining cytoplasm (the "fried-egg" appearance) (**Figure 56–1**).
- Marrow is usually hypercellular, but occasionally hypocellular, mimicking aplastic anemia.
- Immunohistochemistry with CD22 and CD103 antibodies is more sensitive than morphology in detecting residual neoplastic cells in the marrow.
- Diffuse infiltration of splenic red pulp cords and sinuses by hairy cells.
- Ribosomal-lamellar cytoplasmic complexes are seen on electron microscopy in about 50% of patients **(Figure 56–2).**
- Cytoplasm stains strongly positive for tartrate-resistant acid phosphatase (TRAP) in approximately 95% of hairy cell cases. (This classic test has been replaced by flow cytometry for hairy cell markers.)
- Hairy cells most commonly coexpress high levels of CD11c, CD22, CD25, and CD103, but lack of expression of CD21 (**Figure 56–3**).
- Cytogenetic abnormalities in hairy cell leukemia are very diverse and occur in about 50% of

patients, often involving chromosome 5 (eg, trisomy, interstitial deletions, pericentric inversions of chromosome 5).

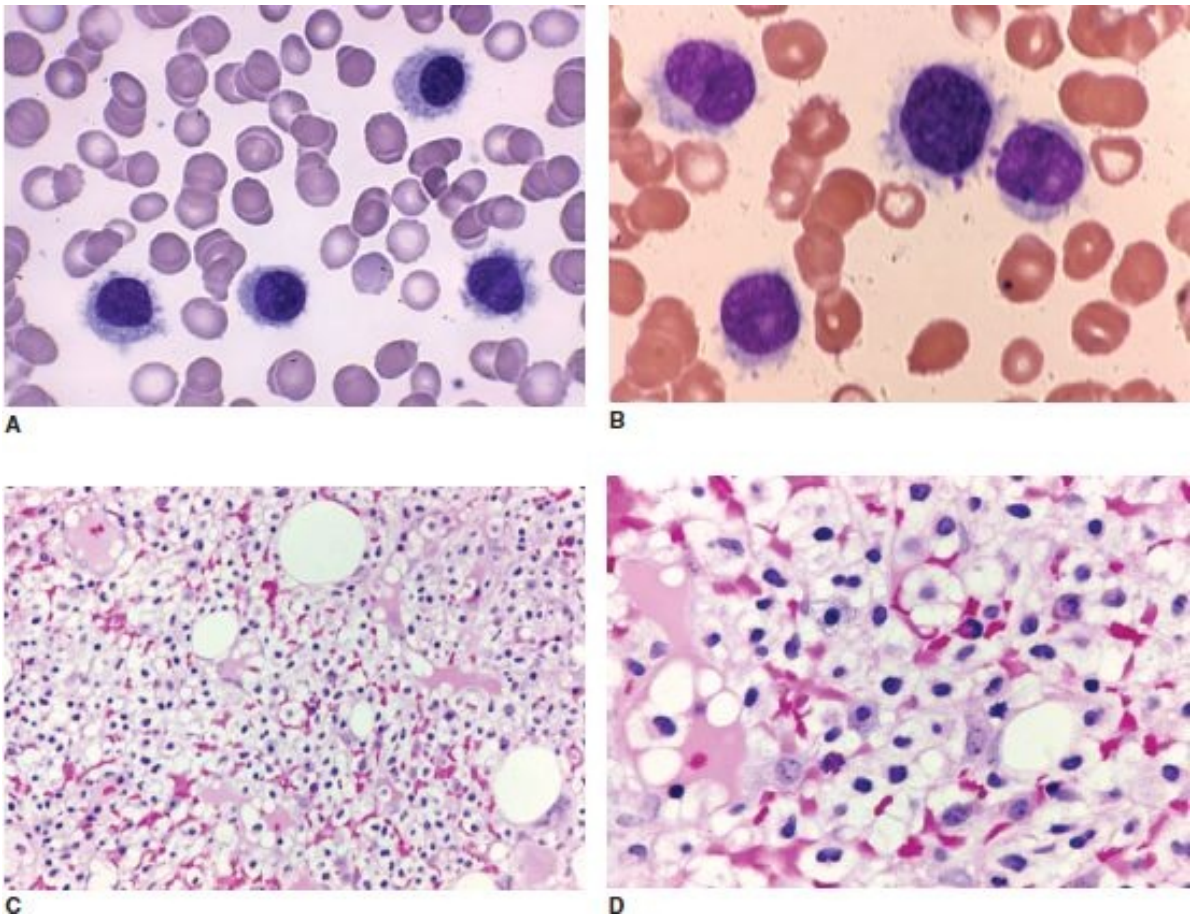


FIGURE 56–1 **A, B.** Two examples of hairy cells in blood. Note irregular surface projections. These surface projections are best seen in wet preparations examined by phase microscopy. **C.** Lower magnification of marrow arrow biopsy in patient with hairy cell leukemia. **D.** Higher magnification of marrow biopsy. Marrow replaced by infiltrate of hairy cells. Note central round nucleus and pale cytoplasm giving the appearance of fried eggs. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

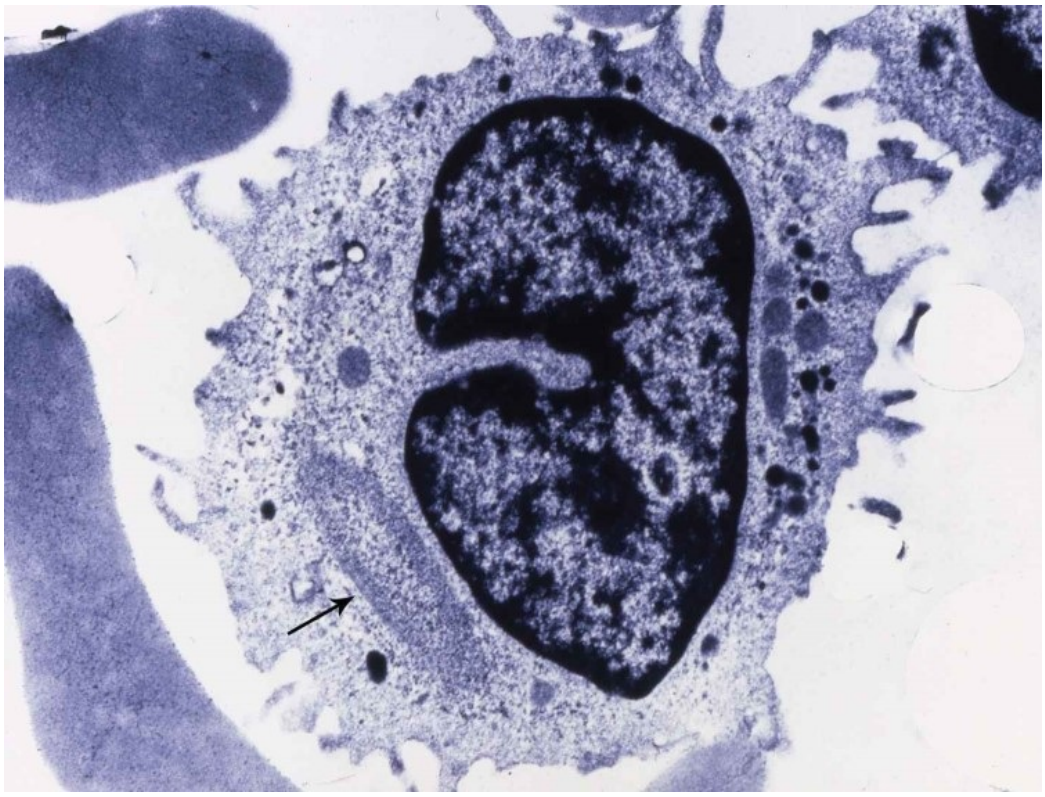


FIGURE 56–2 Hairy cell leukemia. Transmission electron micrograph. Note surface finger-like projections (hairs). An inclusion, a ribosomal-lamellar body cut in longitudinal section, is characteristic of hairy cells (*arrow*). (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

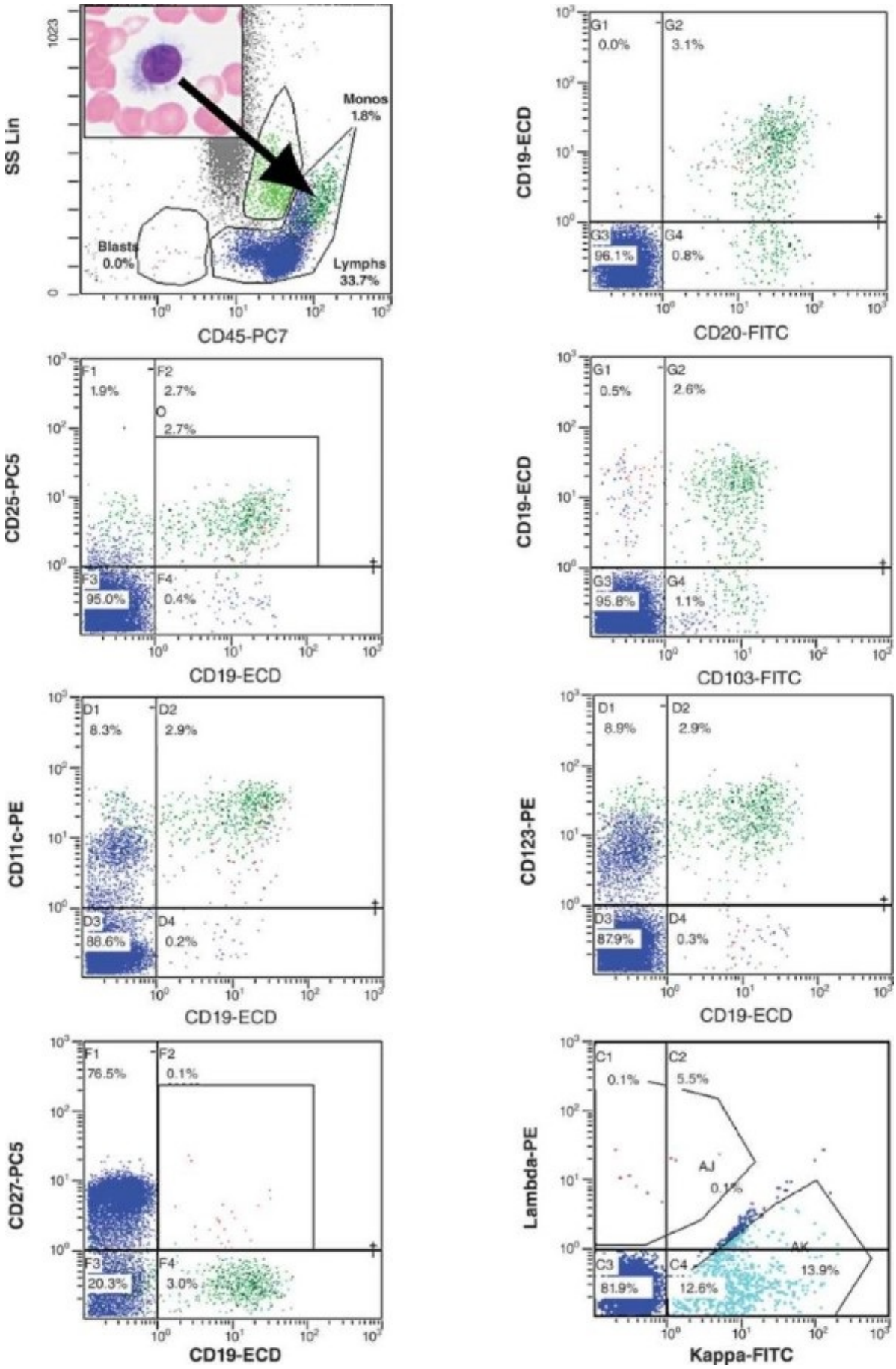


FIGURE 56–3 Hairy cell leukemia (HCL) immunophenotypic profiling should be performed using multiparametric flow cytometry analysis. Both blood and hemodilute marrow aspirates should be analyzed regardless of the number of leukemic cells seen on morphologic review of slides. Because of a very characteristic immunophenotype, definitive diagnosis of HCL can be rendered based on flow cytometric data even with a very low number of leukemic cells. As a result of their complex surface projections,

hairy cells demonstrate moderate to high side scatter characteristics resulting in their shift on flow plots into the region typically occupied by monocytes. Hairy cells are positive for CD11c+, CD19+, CD20+, CD25+, CD103+, CD123+ and show κ light-chain restriction in this example. Hairy cells are negative for CD27–. Very rare cases of HCL may show positive staining for CD5 or CD10 antigens. It is important to note that while atypical, the expression of these antigens does not preclude a definitive diagnosis of HCL in cases with otherwise typical HCL immunophenotype. (Source: *Williams Hematology*, 9th ed, Chap. 93, Fig. 93–1.)

DIFFERENTIAL DIAGNOSIS

- Hairy cell leukemia should be differentiated from nonlymphoid disorders that can present with pancytopenia, splenomegaly, and marrow fibrosis, such as the following:
  — Primary myelofibrosis
  — Mast cell disease
- Hairy cell leukemia can be differentiated from other lymphoproliferative diseases via its clinical and laboratory features (**Table 56–1**).
- Hairy cell leukemia variant:
  — Does not have the *BRAF*^{V600E} mutation
  — More often presents with high white blood cell counts (often > 100×10^9/L)
  — Has a higher nucleus-to-cytoplasm ratio than do hairy cell leukemia cells
  — TRAP staining that is often negative or only weakly positive
  — Not associated with neutropenia or monocytopenia
  — Hairy cell variant cells that are often negative for CD25 and CD103
  — Lack of ribosomal-lamellar complex on electron microscopy
- Other B-cell lymphoproliferative disorders:
  — Chronic lymphocytic leukemia (see Chap. 55)
  — B-cell prolymphocytic leukemia (see Chap. 55)
  — Splenic lymphoma with circulating villous lymphocytes (see Chap. 63)
  — Lymphocytosis, which is more common
  — Lymphocytes that have more basophilic cytoplasm and generally do not express CD103
  — TRAP staining that is either negative or weakly positive

TABLE 56–1 DIFFERENTIAL DIAGNOSIS OF HAIRY CELL LEUKEMIA

Characteristics	HCL	HCL-v	SMZL	SDRPSBL
Number of circulating malignant cells	Low	Moderate	Variable	Low
Monocytopenia	Present	Absent	Absent	Absent
Chromatin	Open	Condensed	Condensed	Condensed
Nucleolus	Absent	Prominent	Absent	Variable
Cytoplasm	Abundant with prominent circumferential hairy projections	Moderate to abundant with variably prominent circumferential hairy projections	Moderate to scant with variably prominent polar hairy projections	Moderate with variably prominent villous projections
Spleen involvement	Red pulp	Red pulp	White pulp	Red pulp
Marrow involvement	Interstitial, diffuse pattern (fried egg morphology)	Sinusoidal may be interstitial	Nodular may be intrasinusoidal	Intrasinusoidal, may be interstitial or nodular

	Marrow reticulin fibrosis			
Marrow reticulin fibrosis	Frequent and marked	Absent	Absent	Absent
Immunophenotype by flow cytometric analysis	CD11c+, CD19+, CD20+ bright, CD22+, CD25+, CD103+,CD123+, FMC7+, kappa or lambda (strong)	CD11c+, CD19+, CD20+, CD22+, CD27+, CD79b+, CD103+, FMC7+, kappa or lambda strong Negative for CD25–, CD123–	CD11c+, CD19+, CD20+, CD22+, CD27+, CD79b+, FMC7+, kappa or lambda strong Negative for CD25–, CD123–	CD11c+/–, CD103+/–, CD19+, CD20+, kappa or lambda+ Negative for CD25–, CD123–
Immunophenotype by immunohistochemistry	DBA44+ AnnexinA1+ Immuno-TRAP+ Cyclin D1+ Faint t-Bet+ V600E BRAF+	DBA44+ Annexin A1– Immuno-TRAP– Cyclin D1– t-Bet– V600E BRAF–	DBA44+/– Annexin A1– Immuno-TRAP– Cyclin D1– t-Bet– V600E BRAF–	DBA44+ Annexin A1– Immuno-TRAP– Cyclin D1– t-Bet– V600E BRAF–
Recurrent mutation	V600E BRAF	None	None	None
Somatic hypermutation of immunoglobulin	> 85% of cases	Mostly	> 50% of cases	Variable

HCL, hairy cell leukemia; HCL-v, variant of hairy cell leukemia; SDRPSBL, splenic diffuse red pulp small B-cell lymphoma; SMZL, splenic marginal zone lymphoma; t-Bet, T-box transcription factor.
Source: *Williams Hematology*, 9th ed, Chap. 93, Table 93–1.

TREATMENT

- Table 56–2 provides guidelines to management of a patient with hairy cell leukemia.
- Approximately 90% of patients require treatment at time of diagnosis. Indications include:
  - — Symptomatic splenomegaly or lymphadenopathy
  - — Anemia (hemoglobin level < 10 g/dL)
  - — Thrombocytopenia (platelet count < 100×10^9/L)
  - — Granulocytopenia (neutrophil count < 1.0×10^9/L) with recurrent bacterial or opportunistic infections
  - — Leukemic phase (white cell count > 20×10^9/L)
  - — Vasculitis
  - — Painful bony involvement
- **Cladribine** (2-chlorodeoxyadenosine) is the treatment of choice for hairy cell leukemia.
  - — This purine analog is given as a 7-day continuous intravenous infusion at 0.1 mg/kg per day. (Successful subcutaneous, oral, and weekly dosing has been reported.) (See **Table 56–2** for alternative schedule of administration.)
  - — It can induce long-lasting complete responses in greater than 75% of patients. Initially, 91% have complete response and 7% a partial response.
  - — Sixteen percent of complete responders have evidence of relapse at 48 months. Approximately 90% of patients initially treated with cladrabine who relapse will have a complete (62%) or partial (26%) response when retreated with the same drug.
  - — In a subsequent study of 207 patients monitored for at least 7 years after cladrabine treatment, 95% had achieved a complete response and 5% a partial response after a single

7-day course. The overall survival at 108 months was 97% and the median disease-free duration for all responders was 98 months.
— Notable toxicities of cladrabine:
  - Aseptic fever in setting of neutropenia
  - T-cell depletion, particularly $CD4^+$ cells

- **Pentostatin** (2′-deoxycoformycin):
  — This purine analog inhibits adenosine deaminase.
  — It is a good second choice drug for patients unresponsive or refractory to cladrabine.
  — The drug is administered as an intravenous bolus of 4 mg/m^2 every other week for 3 to 6 months until maximum response as judged by decrease of blood and marrow hairy cells, reduced spleen size, and improvement in normal blood cell counts.
  — Complete response rates with pentostatin (~50%) are lower than that achieved with cladribine.
  — Pentostatin may not be effective in patients refractory to cladribine.
  — Notable toxicities:
    - Fever, rash, and conjunctivitis
    - Reversible renal dysfunction
    - Mild hepatic toxicity
    - Depletion of $CD4^+$ cells
- **Interferon**-α (IFN-α):
  — Complete response rate is 8%; 74% achieve a partial response.
  — IFN is not curative; 50% relapse less than 2 years after treatment.
  — Usual dosage schedule is 2×10^6 U IFN-α_{2b}/m^2 subcutaneously three times weekly for 12 months or 3×10^6 U IFN-α_{2a}/m^2 subcutaneously daily for 6 months and decreased to 3 times per week for an additional 6 months.
  — IFN is not as effective as purine analogs.
  — Toxicity:
    - Flu-like symptoms (fever, myalgia, malaise)
    - Myelosuppression
- **Rituximab**
  — Hairy cells express CD20 and thus an anti-CD20 monoclonal antibody is rational.
  — The responses have been modest but it should be considered in patient's refractory to cladrabine and pentostatin.
  — Administration is 375 mg/m^2 intravenously weekly for 4 to 8 weeks.
  — A proportion (25%–75%) of patients has a complete or partial remission, which may be sustained for several years in a proportion of responders. Others relapse and progress.
- **Anti-CD22 Immunotoxin BL22**
  — Anti-CD22 is fused to a *Pseudomonas* exotoxin.
  — It can induce remissions in a high proportion of patients who are refractory to cladrabine.
  — It may be associated with a reversible hemolytic uremic syndrome in a minority of patients.
  — Vemurafenib is a BRAF inhibitor to which patient's in relapse have a high response rate.
- **Splenectomy**
  — This procedure is not curative.

— Current indications:
  - Massive, painful, and/or ruptured spleen
  - Pancytopenia and an active infection with opportunistic pathogen (eg, *Mycobacterium*). Splenectomy usually results in marked increase in neutrophil and monocyte count and better response to antimicrobial treatment
  - Failure of systemic chemotherapy
- **Granulocyte colony-stimulating factor** (G-CSF):
  — This may ameliorate neutropenia.
  — It is an adjunct to therapy in cases of infection.
- **Radiation**
  — Lytic bone lesions can be treated with low-dose irradiation.

TABLE 56–2 MANAGEMENT OF HAIRY CELL LEUKEMIA

Determine accurate diagnosis
• Marrow biopsy with immunohistochemical analysis
• Blood immunophenotypic characterization
Decision on initiation of therapy
• Approximately 10% can be carefully followed on "watch and wait" approach but majority of patients require treatment
• Determinants or symptoms prompting treatment: symptomatic splenomegaly or laboratory studies showing absolute neutrophil count < 1000/μL, hemoglobin < 10 g/dL, or platelet count < 100,000/μL
Important assessments before therapy for leukemia
• Presence or suspicion for infection
• Adequate renal function
• Previous exposure to hepatitis
Decision on front-line therapy
• Cladribine 0.1 mg/kg/d for 7 days continuous intravenous infusion
• Cladribine 0.12 mg/kg/d for 5 days as 2-hour intravenous infusion vs weekly infusion for 6 weeks
• Pentostatin 4 mg/m^2 intravenous dose every 2 weeks until maximal response or failure
Assessment of response
• Following induction therapy, a marrow biopsy to document quality of response and quantitate minimal residual disease (MRD).
• Methods for quantification of MRD with immunohistochemical stains and the optimal timing for MRD assessment are under investigation.
• In general, response assessment after cladribine is recommended after 3 to 5 months. In contrast, response assessment following pentostatin is made at time of best clinical response.
Clinical investigations for resistant hairy cell leukemia
• Alternate purine analogs alone or combined chemoimmunotherapy (eg, bendamustine and rituximab)
• Immunotoxin conjugates (eg, moxetumomab pasudotox [HA22])
• BRAF V600E inhibitors (eg, vemurafenib)

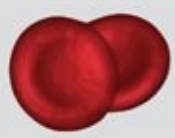
Source: *Williams Hematology*, 9th ed, Chap. 93, Table 93–2.

CLINICAL COURSE

- With cladribine treatment, patients may be considered curable, with progression free survival rates as high as of 95% at 4 years.
- Remissions of over 10 years duration are common.
- A plateau in relapse has not been reached at over 10 years of remission and, thus, a risk of late relapse exists.
- Minimal residual disease can be found using immunohistochemistry and/or flow cytometry in about 35% of long-term responders (median disease-free survival 16 years), but its presence

does not necessarily predict for relapse because very long-term responders are as likely as not to have minimal residual disease by very sensitive techniques to identify marrow hairy cells. It is not clear that immediate therapy improves results.

- Relapsed patients after first treatment with cladrabine have a high response rate to retreatment with cladrabine or another agent.
- Infections, including by opportunistic organisms, that were the cause of death in more than 50% of patients prior to the availability of cladrabine, are now uncommon.



For a more detailed discussion, see Michael R. Grever and Gerard Lozanski: Hairy Cell Leukemia, Chap. 93 in *Williams Hematology*, 9th ed.

CHAPTER 57
Large Granular Lymphocytic Leukemia

DEFINITION

- T-cell large-granular lymphocytic (T-LGL) leukemia results from the clonal expansion of large granular lymphocytes (LGLs) with a T-cell (CD3+) phenotype and a clonal T-cell receptor gene rearrangement(s).
- Natural killer (NK)-LGL leukemia is a clonal expansion of LGL with a NK cell (CD3–) phenotype. It lacks convenient markers to determine clonality, such as antigen receptor rearrangements. (See Chap. 66.)

T-LGL LEUKEMIA

Etiology and Pathogenesis

- There is suggestive evidence of a role for human T lymphotropic virus (HTLV) retroviral infection in some patients.
- Most patients are not infected with either HTLV-I or HTLV-II.
- Cytomegalovirus implicated in rare cases of CD4+ T-LGL.
- Leukemic cells have features of antigen-activated cytotoxic T lymphocytes, suggesting a role for antigen in initial LGL expansion.
- Constitutive overexpression of the Fas ligand (CD178), which also is found at high levels in patients' sera, may be a factor in many disease manifestations (eg, neutropenia, rheumatoid arthritis).

Clinical Features (Table 57–1)

TABLE 57–1 CLINICAL FEATURES OF T-CELL LARGE GRANULAR LYMPHOCYTIC LEUKEMIA

	Pandolfi (1990)	Loughran (1993)	Dhodapkar (1994)	Semenzato (1997)	Neben (2003)	Bareau (2010)
Number of patients	151	129	68	162	44	201
Median age	55	57	61	59	63	59
Male/Female	1.3	0.8	1	0.8	1.0	0.8
Symptomatic	72%	–	69%	–	73%	82%
Splenomegaly	50%	50%	19%	50%	35%	24%
Hepatomegaly	34%	23%	1%	32%	–	10%
Adenopathy	13%	1%	3%	13%	5%	6%
B symptoms	–	–	12%	–	–	7%

Infections	38%	39%	15%	56%	–	23%
Rheumatoid arthritis	12%	28%	26%	36%	20%	17%
Rheumatoid factor	–	57%	61%	43%	48%	41%
Antinuclear antibodies	–	38%	44%	38%	48%	48%
Autoimmune cytopenias	–	–	7%	9%	5%	7%
Lymphocytosis				29%		
LGL > 4×10^9/L	52%	52%	–	–	–	14%
LGL 1–4×10^9/L	38%	40%	–	–	–	50%
LGL < 1×10^9/L	10%	8%	–	7%	–	36%
Neutropenia						
Moderate (< 1.5×10^9/L)	64%	84%	74%	–	52%	61%
Severe (0.5×10^9/L)	7%	48%	40%	37%	41%	26%
Anemia						
Any severity	25%	49%	51%	26%	89%	24%
Severe (Hgb < 8 g/dL)	37%	–	19%	–	36%	7%
Thrombocytopenia	9%	19%	20%	29%	36%	19%
LGL marrow infiltration	67%	88%	–	76%	83%	72%
Hypergammaglobulinemia	–	45%	5%	43%	–	35%
Monoclonal gammopathy	–	45%	8%	–	–	10%
Need for treatment	30%	73%	69%	33%	80%	44%
LGLL-related death	14%	36%	8%	27%	–	7%

Hgb, hemoglobin; LGL, large granular lymphocyte; LGLL, large granular lymphocytic leukemia.
Modified with permission from Bareau B, Rey J, Hamidou M, et al. Analysis of a French cohort of patients with large granular lymphocyte leukemia: A report on 229 cases. *Haematologica*. 2010;95(9):1534–1541.
Source: *Williams Hematology*, 9th ed, Chap. 94, Table 94–1.

- About half of patients have palpable splenomegaly.
- About one-third of patients have recurrent bacterial infections.
- "B symptoms" (eg, low-grade fevers, night sweats, and/or weight loss) (aggressive variant) are very infrequent.
- About one-fourth of patients have rheumatoid arthritis, often with features of "Felty syndrome."
- Less than one-tenth of patients have lymphadenopathy.

Laboratory Features

- Approximately half of the patients have anemia, often caused by pure red cell aplasia and/or autoimmune hemolytic anemia.
- Approximately one-fifth of patients have thrombocytopenia.
- Nearly three-fourths of patients have neutropenia, often less than 0.5×10^9/L.
- About one-fourth of patients do not have increased blood total lymphocyte counts.
- The median LGL count in patients is 4.0×10^9/L (normal mean: 0.3×10^9/L) (**Figure 57–1**).
- Immunophenotype of LGL cells in blood and marrow: CD3+CD8+CD16+CD57+CD4–

CD56–, and, often, HLA-DR+.

- LGL cells have clonal T-cell–receptor gene rearrangement(s), usually involving α and β chains.
- Patients commonly have elevated levels of certain autoantibodies and other serologic abnormalities (see **Table 57–1**).
- More than 90% of patients have LGL infiltration of the marrow and splenic red pulp.
- Marrow infiltration may be nodular or interstitial. If interstitial, it may be difficult to appreciate involvement without staining for neoplastic cells using immunocytochemistry (**Figure 57–2**).

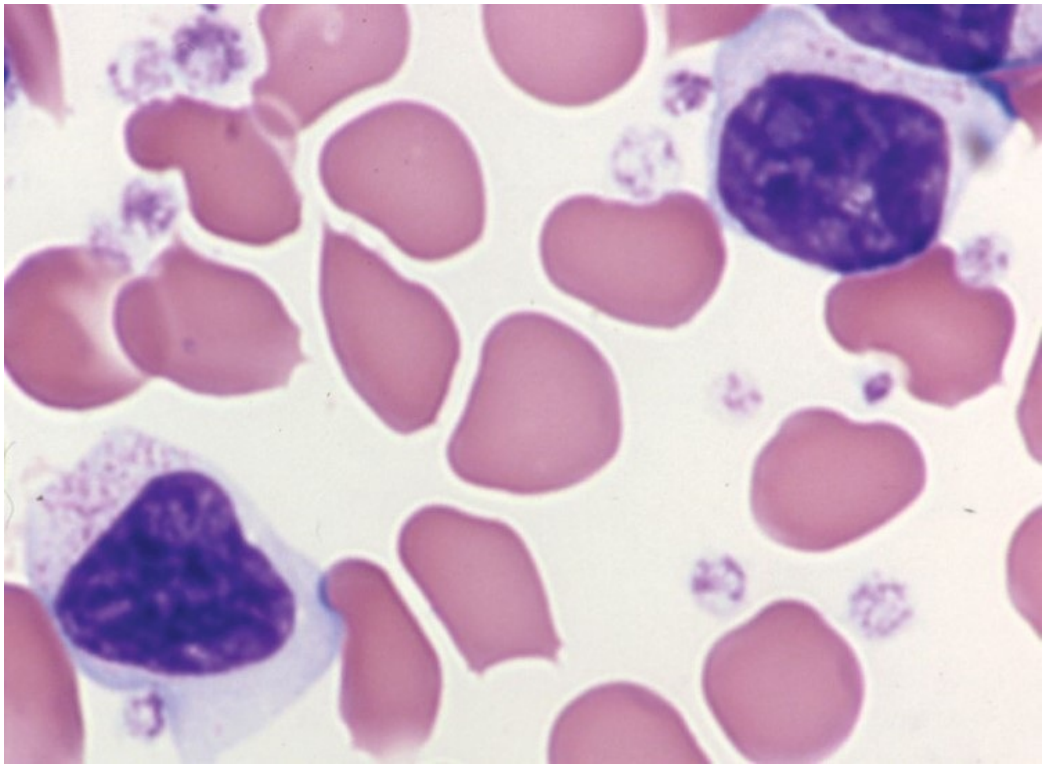


FIGURE 57–1 Buffy coat blood film. High magnification image of two large granular lymphocytes (LGLs) displaying larger size than characteristic blood small lymphocytes and with more cytoplasm. Cytoplasmic granules are conspicuous in both LGLs. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

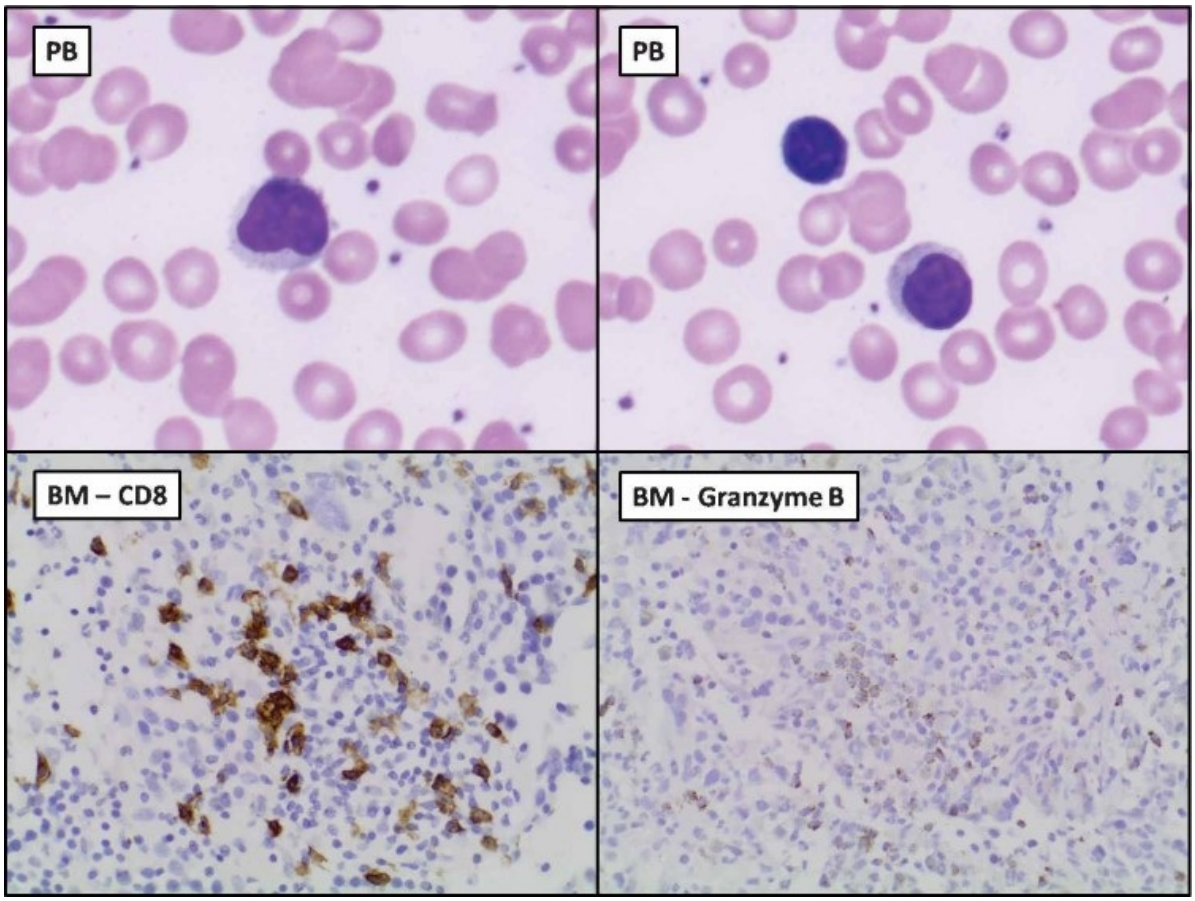


FIGURE 57–2 Morphology and immunohistochemical analysis of T-cell large-granular lymphocytic (T-LGL) leukemia in peripheral blood (PB) and bone marrow (BM). Upper panels show high-power images of circulating large granular lymphocytes (LGLs) from a patient with T-LGL leukemia (original magnification = 500×). Note for comparison the small lymphocyte next to the LGL in the right panel. LGLs are slightly larger than other lymphocytes, and they have more nuclear membrane irregularity, moderate amounts of pale blue cytoplasm, and fine cytoplasmic granules. The lower panels show foci of atypical clusters of CD8+ (left) and granzyme B+ (right) lymphocytes in the bone marrow of a patient with T-LGL leukemia (original magnification = 500×). The identification of atypical clusters of at least eight CD8+ and/or at least six granzyme B+ lymphocytes supports the diagnosis of LGL leukemia in the appropriate clinical setting. (Source: *Williams Hematology*, 9th ed, Chap. 94, Fig, 94–1.)

Differential Diagnosis

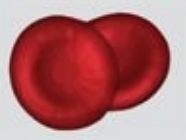
- T-LGL leukemia should be considered in patients with increased blood LGL counts and the following:
  — Chronic or cyclic neutropenia
  — Pure red cell aplasia
  — Rheumatoid arthritis
- T-LGL leukemia can be distinguished from NK-LGL leukemia by immunophenotype and clonal T-cell–receptor gene rearrangement. NK-LGL leukemia is a much more aggressive disease.
- Hepatosplenic T-cell lymphoma typically occurs in young men and follows a more aggressive course. (See Chap. 66).

Therapy, Course, and Prognosis

- This disease is usually chronic, and treatment is not always indicated.
- Unusual cases that coexpress CD3 and CD56 may have a more aggressive clinical course.
- Significant morbidity/mortality from infections occurs.
- Indications for therapy include (1) severe neutropenia or moderate neutropenia and infection,

(2) transfusion-dependent anemia, (3) platelet count < 50 × 10^9/L, and (4) coincident autoimmune diseases requiring therapy.

- Low-dose methotrexate 10 mg/m^2, orally, once weekly or cyclophosphamide 100 mg, orally, daily, or cyclosporine may be effective in alleviating neutropenia/anemia.



For a more detailed discussion, see Pierluigi Porcu and Ahron G. Freud: Large Granular Lymphocytic Leukemia, Chap. 94 in *Williams Hematology*, 9th ed.

CHAPTER 58

General Considerations of Lymphoma: Epidemiology, Etiology, Heterogeneity, and Primary Extranodal Disease

EPIDEMIOLOGY

- Approximately 72,000 cases of non-Hodgkin lymphoma (NHL) will be diagnosed in 2015 and approximately 20,000 persons will die of the disease in the United States.
- NHL represents approximately 4.5% of cancers in the United States and 3.5% of cancer deaths, annually.
- The age-adjusted incidence rates per 100,000 persons for NHL in the United States are 25 for white males, 18 for black males, 17 for white females, and 12 for black females.
- The risk of NHL in the United States is approximately three times that of several underdeveloped countries and two times that of several comparable industrialized countries.
- There is a logarithmic increase in annual incidence in both men and women from late teenagers to octogenarians in the United States: males 15 to 19 years of age, 2.3 cases/100,000 persons; males 80 to 84 years of age, 147 cases/100,000 persons; females 15 to 19 years, 1.2 cases/100,000 persons; and females 80 to 84 years, 102 cases per 100,000 persons.
- Follicular lymphoma represents approximately 25% of NHL cases in the United States but is uncommon in many developing countries and in Asia, especially China and Japan.
- Diffuse large B-cell lymphoma represents approximately 30% of NHL cases in the United States.
- The annual incidence of NHL, but not Hodgkin lymphoma, increased significantly between 1972 and 1995 in the United States and western European countries. The increase probably started before 1972 based on European data, but the United States National Cancer Institute did not track specific-site cancer incidence before that date.
- The rate is still increasing slightly for women and older men in the United States. Orbital adnexal lymphoma and mantle cell lymphoma are increasing at a rate of approximately 5% per year.
- The evidence that benzene, other solvents, pesticides, herbicides, dyes, various occupations, and other industrial exposures increase the relative risk of lymphoma is disputed and, at this time, is insufficient to reach a level of medical or scientific certainty, according to the International Agency on Research in Cancer.
- There are instances of familial clustering and an increase in the relative risk of lymphoma in siblings of patients with lymphoma or related hematologic malignancies (eg, myeloma). These so-called nonsyndromic examples of increased familial risk are likely explained by as yet undefined predisposition genes, akin to the Li-Fraumeni syndrome, which is the result of germline inheritance of mutated p53.
- Several syndromic immunodeficiency states increase the relative risk of lymphoma in family

members (see “Immunosuppression and Autoimmunity,” below).

HISTOLOGIC HETEROGENEITY

- The World Health Organization has categorized more than 30 unique histopathologic types of NHL and these are shown in **Table 58–1** with their approximate relative frequency. Approximately 88% are B-cell lymphomas and approximately 12% are T-cell lymphomas. There are striking variations in the incidence of various subtypes of NHL in different geographic areas throughout the world.

TABLE 58–1 HISTOLOGIC SUBTYPES AND RELATIVE FREQUENCY OF THE NON-HODGKIN LYMPHOMAS (NHL)

A. B-cell lymphomas (~88% of all non-Hodgkin lymphoma [NHL])
   1. Diffuse large B-cell lymphomas (30%)
      a. T-cell–rich large B-cell lymphoma
      b. Primary diffuse large B-cell lymphoma of the central nervous system
      c. Primary cutaneous diffuse large B-cell lymphoma
      d. Epstein-Barr virus (EBV)–positive diffuse large B-cell lymphoma of the elderly
      e. Diffuse large B-cell lymphoma arising in human herpesvirus (HHV)-8–associated multicentric Castleman disease
      f. Diffuse large B-cell lymphomas with features simulating Hodgkin lymphoma
   2. Follicular lymphoma (25%)
   3. Extranodal marginal zone lymphoma of mucosa-associated lymphatic tissue (MALT lymphoma) (7%)
   4. Small lymphocytic lymphoma-chronic lymphocytic leukemia (7%)
   5. Mantle cell lymphoma (5%)
   6. Primary mediastinal (thymic) large B-cell lymphoma (3%)
   7. Lymphoplasmacytic lymphoma–Waldenström macroglobulinemia (< 2%)
   8. Nodal marginal zone B-cell lymphoma (< 1.5%)
   9. Splenic marginal zone lymphoma (< 1%)
   10. Extranodal marginal zone B-cell lymphoma (< 1%)
   11. Intravascular large B-cell lymphoma (< 1%)
   12. Primary effusion lymphoma (< 1%)
   13. Primary cutaneous follicle center lymphoma (1%)
   14. Burkitt lymphoma–Burkitt leukemia (1.5%)
   15. Plasmablastic lymphoma (< 1%)
   16. Lymphomatoid granulomatosis (< 1%)

B. T-cell and natural killer (NK)–cell lymphomas (~12% of all NHL)
   1. Extranodal T- or NK-cell lymphoma
   2. Enteropathy-associated T-cell lymphoma
   3. Hepatosplenic T-cell lymphoma
   4. Subcutaneous panniculitis-like T-cell lymphoma
   5. Cutaneous T-cell lymphoma (Sézary syndrome and mycosis fungoides)
   6. Primary cutaneous γδT-cell lymphoma
   7. Anaplastic large cell lymphoma
   8. Angioimmunoblastic T-cell lymphoma
   9. Primary T-cell lymphoma unspecified

C. Immunodeficiency-associated lymphoproliferative disorders (see Table 58–2 for inherited diseases associated with immunodeficiencies and lymphoma)
   1. HIV-associated lymphoma
   2. Post-transplantation lymphoproliferative disorder
   3. Lymphoma associated with a primary immune disorder

This table is compiled from information presented in the *World Health Organization's Classification of Tumors of Hematopoietic and Lymphoid Tissues*. The parenthetical percentages are approximate but give some sense of the relative distribution of subtypes. The frequency of lymphoma varies depending on the geographic area under consideration. The frequencies cited here are approximate and related to those observed in the United States, the United Kingdom, or western

Europe. Rare subtypes are not listed.
Source: *Williams Hematology*, 9th ed, Chap. 95, Table 95–1.

EFFECT OF GENE POLYMORPHISMS

- Single nucleotide polymorphism base analysis has indicated that lymphomagenesis may be linked to polymorphic genes that are involved in apoptosis, cell cycle regulation, lymphocyte development, and inflammation. The polymorphisms could also be linked to individual susceptibility to certain environmental exposures.

INFECTIOUS AGENTS

- Adult T-cell leukemia-lymphoma is caused by human T-cell lymphocytotrophic virus-(HTLV)-1 (see Chap. 66).
- The Epstein-Barr virus genome has been found with a high prevalence in the neoplastic lymphocytes of African Burkitt lymphoma, post-transplantation lymphoma, human immunodeficiency virus (HIV)–related lymphoma, primary central nervous system lymphoma, primary effusion lymphoma, immunoblastic plasmacytoid B-cell lymphoma, oral cavity plasmablastic lymphoma, and extranodal natural killer (NK) cell/T-cell lymphoma. The precise role of this virus in lymphomagenesis has not been defined, but it is likely to be an important facilitating factor in some of these lymphoma types.
- Human herpesvirus-8 is associated with Kaposi sarcoma, Castleman disease, and primary effusion lymphoma found most frequently in immunodeficiency associated with HIV infection.
- Hepatitis B and C virus have been implicated in the pathogenesis of lymphoma based on the seropositivity of cases compared with controls. Hepatitis C virus has a predilection for lymphocytes, and it has been specifically associated with diffuse large B-cell lymphoma, marginal zone lymphoma, and lymphoplasmacytic lymphoma, but not follicular lymphoma. The etiologic role of and the pathogenetic mechanisms attributed to these viruses have not been established.
- *Helicobacter pylori* can cause marginal zone B-cell lymphomas of mucosa-associated lymphatic tissue (MALT lymphoma), notably of the stomach. This organism is the first bacterium shown to be capable of inducing a human neoplasm (see Chap. 63).
- *Chlamydophila psittaci* has been associated with a majority of cases of a specific extranodal mucosa-associated lymphoid tissue lymphoma, ocular adnexal lymphoma.
- *Campylobacter jejuni* and *Borrelia burgdorferi* have been associated with immunoproliferative diseases of the small intestines and B-cell lymphoma of the skin.

IMMUNOSUPPRESSION AND AUTOIMMUNITY

- A number of inherited immunodeficiency syndromes listed in **Table 58–2** are associated with a predisposition to lymphoma.
- Acquired immunodeficiency states, including acquired immunodeficiency syndrome (AIDS)–related lymphoma and post-transplantation–related lymphoma, usually have a B-cell lineage

immunophenotype and often involve extranodal sites (eg, skin or central nervous system), are aggressive in behavior, and often associated with Epstein-Barr virus infection of the neoplastic B lymphocytes.

- The occurrence of Ig (V) mutations in acquired immunodeficiency-related lymphoma strongly suggests a germinal center B lymphocyte transformation.
- The incidence and severity of immunodeficiency-related lymphoma have increased in relationship to the use of more powerful immunosuppressive agents, such as cyclosporine and in the setting of mismatched T-cell–depleted allogeneic hematopoietic stem cell grafts.
- Several autoimmune disorders are associated with an increased relative risk of lymphoma. These include systemic lupus erythematosus, Sjögren syndrome, autoimmune thyroid disease, and, perhaps, rheumatoid arthritis. For example, lymphoma is increased 6.5-fold and MALT lymphoma of the parotid gland 1000-fold in Sjögren syndrome.

TABLE 58–2 INHERITED SYNDROMES PREDISPOSING TO LYMPHOMA

	Altered Genes			
Syndrome	**Inheritance**	**Description**	**Mechanism**	**Leukemia Type**
DNA repair defects				
Ataxia telangiectasia	R	*ATM* homozygotes Dominant-negative missense mutations	Genomic instability Increased translocations in T cells formed at the time of V(D)J recombination	T cell lymphoma, T-cell ALL, T-cell PLL, B-cell lymphoma
Bloom	R	*BLM*	Genomic instability	ALL, lymphoma
Nijmegen breakage	R	*NBS1*	Genomic instability Altered telomere maintenance	Lymphoid tumors, especially B-cell lymphoma
Tumor-suppressor gene defect				
Li-Fraumeni*	D	*p53*	Defect in tumor suppressor	CLL, ALL, Hodgkin and Burkitt lymphoma
Immunodeficiency states				
Common variable immunodeficiency	R and D	Defect in CD40 signaling	Failure of B-cell maturation	Burkitt, MALT, other B-cell lymphomas, Hodgkin lymphoma
Severe combined immunodeficiency disease (SCID)	R	*ADA*	Defective T-cell + B-cell function	B-cell lymphoma
Wiskott-Aldrich	X	*WASP*	Signaling and apoptosis	Hodgkin and non-Hodgkin lymphoma
X-linked immunodeficiency with normal or increased IgM	X	*CD40L*	CD40 ligand defect on T cell	Hodgkin and non-Hodgkin lymphoma
X-linked lymphoproliferative syndrome (XLP)	X	*SAP*	Defect in immune signaling	EBV-related B-cell lymphoma
Apoptotic defect				
Autoimmune	D	*APT (FAS)*	Germline heterozygous	Lymphoma

lymphoproliferative syndrome (ALPS)			*FAS* mutations; defective apoptosis	
Unknown defect				
Dubowitz	R	Unknown	Unknown	ALL, lymphoma
Poland	D	May not be inherited	Unknown	ALL, lymphoma
Wilms tumor (WT)	D	Unknown	Unknown	ALL, Castleman disease

ALL, acute lymphocytic leukemia; CLL, chronic lymphocytic leukemia; D, dominant; EBV, Epstein-Barr virus; MALT, mucosa-associated lymphatic tissue lymphoma; R, recessive; T-PLL, T prolymphocytic leukemia; X, X-linked.

*Li-Fraumeni or Li-Fraumeni–like syndrome has been described in which a gene other than p53 is mutated. hCHK2 in particular has been described as etiologic. We have not included these variants in the table because we are uncertain if lymphoma is one of the cancers for which susceptibility is increased.

Source: *Williams Hematology*, 9th ed, Chap. 95, Table 95–2.

SPECIFIC CHROMOSOME ABNORMALITIES AND HISTOLOGIC SUBTYPE

- Chromosome abnormalities involving all 22 autosomes and the sex chromosomes can occur with lymphoma.
- Lymphomas have a high frequency of fusion genes usually one of two types: oncogenes activated by juxtaposing with immunoglobulin or T cell receptor genes or by forming chimeric genes that activate mutant kinases or transcription factors.
- Approximately 85% of cases of follicular lymphoma have a t(14;18)(q32;q21)(*IgH;BCL-2*) in the lymphoma cells. Presumably the overexpression of BCL-2 contributes to an antiapoptotic effect favoring an accumulation of long-lived centrocytes (see Chap. 61).
- In patients with Burkitt lymphoma, t(8;14)(q24;q32), principally, or t(2;8)(p13;q24) or t(8;22)(q24;q11) is present in the lymphoma cell. The common feature is the formation of a fusion gene involving the *MYC* gene at band q24 on chromosome 8 with either the *IgH* or *Ig*κ or *Ig*λ genes (see Chap. 64).
- The t(2;5)(p23;q35) in the cells of anaplastic large cell lymphoma (ALCL) involves the *NPM* gene at 5p35 and the ALCL tyrosine kinase (*ALK*) gene at 2p23, resulting in a novel oncoprotein, p80. The translocation occurs in about 50% of cases in adults and a higher proportion of children.
- Four translocations, t(11;18)(*API2;MALT1*), t(1;14)(*IgH-BCL10*), t(14;18)(*IgH;Malt1*), and t(3;14)(*IgH;FOXP1*) have been associated with marginal zone lymphomas of the MALT type at different sites. In the first three translocations shown, the oncogenic product targets the nuclear factor-κB pathway (see Chap. 63).
- Most cases of mantle cell lymphoma have t(11;14)(q13;q32) in the lymphoma cells. This translocation juxtaposes CCND1 and the IgH genes and results in upregulation of cyclin D1, used as a marker in the diagnosis of this disease (see Chap. 62).

GENERAL APPROACHES TO LYMPHOMA MANAGEMENT

- Complete history and physical examination to determine extent of superficial

lymphadenopathy, evidence of extranodal involvement, and presence of B symptoms (fever to > 38°C, night sweats, weight loss > 10% body weight in past 6 months).

- Staging should be carried out to determine extent of disease as shown in **Table 58–3**.
- The Lugano staging system for lymphoma is shown in **Table 58–4**.

TABLE 58–3 STAGING PROCEDURES FOR LYMPHOMA

Initial studies
History and physical examination
Complete blood count
Metabolic panel including renal and hepatic function
Uric acid
Lactate dehydrogenase and/or β2-microglobulin
Hepatitis B and C serologies (if rituximab therapy planned)
HIV serology Tumor biopsy specimen with histopathology
Flow cytometry of tumor specimen
Immunohistochemistry of tumor specimen
Cytogenetic analysis (including iFISH for lymphoma-associated translocations)
PET/CT scans of neck, chest, abdomen, and pelvis (for FDG-avid lymphomas)
Contrast-enhanced CT scans of neck, chest, abdomen, and pelvis (particularly for lymphomas that are not FDG-avid)
Additional studies (useful in selected cases)
Marrow aspiration and biopsy
Pregnancy testing in women of childbearing potential
Immunoglobulin and *TCR* gene rearrangement studies
Cardiac ejection fraction measurement (if anthracycline therapy planned)
Magnetic resonance imaging of brain if neurologic signs or symptoms
Cerebrospinal fluid analysis (including flow cytometry) for high-risk aggressive lymphomas or if neurologic signs or symptoms are present
Gastrointestinal studies (imaging and endoscopy) if Waldeyer ring involvement, mantle cell lymphoma, or enteropathy associated lymphoma

CT, computed tomography; FDG, 2-fluorodeoxyglucose; iFISH, interphase fluorescence in situ hybridization; PET, positron emission tomography; TCR, T-cell receptor.

Source: *Williams Hematology*, 9th ed, Chap. 95, Table 95–3.

TABLE 58–4 THE LUGANO STAGING SYSTEM FOR LYMPHOMAS

Stage	Involvement*	Extranodal (E) Status
Limited		
I	One nodal group involved	Single extranodal lesions without nodal involvement
II	Two or more nodal groups involved, on the same side of the diaphragm	Stage I or II nodal involvement with limited, contiguous extranodal extension
II bulky†	As in II above, but with "bulky" disease	Not applicable
Advanced		
III	Involvement of nodal groups on both sides of the diaphragm‡	Not applicable
IV	Diffuse involvement of a visceral organ not contiguous with an involved nodal site	Not applicable

CT, computed tomography; DLBCL, diffuse large B-cell lymphoma; FDG, 2-fluorodeoxyglucose; HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphoma; PET, positron emission tomography.

*Extent of disease is assessed by PET/CT imaging for FDG-avid lymphomas and by CT imaging for nonavid histologies.

†A nodal mass of ≥ 10 cm, or greater than one-third of the transthoracic diameter at any level of thoracic vertebrae as determined by CT imaging is considered bulky disease for HL. There is no consensus on the size of "bulk" for NHL with a suggestion that 6

cm may be optimal for follicular lymphoma. Sizes between 6 cm and 10 cm have been advocated to define bulk for DLBCL. Current recommendations are to record the longest measurement by CT scan and not use the "X" notation to designate bulky disease. Stage II bulky disease may be considered to be either limited or advanced disease depending on histology and associated prognostic factors. Note that B type symptoms are not considered important in this staging stratification.

‡Tonsils, Waldeyer ring, and spleen are considered nodal tissue in this staging system.

Source: *Williams Hematology*, 9th ed, Chap. 95, Table 95–4.

PRIMARY EXTRANODAL LYMPHOMA

- Lymphomas involving extranodal sites most commonly occur simultaneously with nodal involvement at time of diagnosis or during course of the disease.
- Lymphoma only found in extranodal sites after staging procedures (see **Table 58–4**) is called *primary extranodal lymphoma*.
- Solitary extranodal lymphoma can occur in any organ. Lymphoma should be considered in the differential diagnosis of any solitary mass.
- The histology of primary extranodal lymphoma is usually marginal zone lymphoma of mucosa associated lymphoid tissue or diffuse large B-cell lymphoma. Other lymphoma types may occasionally be the histologic diagnosis.
- Therapy is usually a combination of excision, radiotherapy, multidrug chemotherapy, and a lymphocyte-directed monoclonal antibody. Rituximab, cyclophosphamide, hydroxydoxorubicin (Adriamycin), vincristine (Oncovin), and prednisone (R-CHOP) is one commonly used regimen for extranodal B-cell malignancies.
- The pathobiology underlying a propensity of primary extranodal lymphoma to arise simultaneously in paired organs (kidneys, ovaries, breasts, eyes, adrenals, and others) is unknown.
- Specific anatomical sites include:
  — *Bone*. Primary lymphoma may affect any bone but usually the long bones are involved. If the skull is involved, central nervous system invasion may occur.
  — *Breast*. Primary lymphoma of the female breast mimics carcinoma. Staging finds lymph node, marrow, or other extranodal sites of involvement in half the cases.
  — *Central nervous system*. Involvement of the leptomeninges may produce headache, stiff neck, and cranial nerve impairment. Brain involvement can result in headache, lethargy, papilledema, focal neurologic signs, or seizures. Spinal cord involvement can result in back pain, extremity weakness, paresis, and paralysis. This is a usually aggressive type of diffuse large B-cell lymphoma. Intracerebral lymphoma is a feature of AIDS.
  — *Chest and lung*. Primary pulmonary lymphoma may present as a solitary mass in the lung and require lung biopsy for diagnosis. Primary chest wall lymphoma can be accompanied by fever, sweating, and dyspnea and require excisional biopsy for diagnosis. Primary endobronchial lymphoma may follow lung transplantation and cause airway obstruction.
  — *Endocrine glands*. Primary adrenal lymphoma usually presents bilaterally and may lead to adrenal insufficiency. Primary thyroid lymphoma often develops on the background of autoimmune (Hashimoto) thyroiditis. Primary pituitary lymphoma can result in pituitary insufficiency, including diabetes insipidus.
  — *Eye*. Ophthalmic lymphoma is the most common orbital malignancy and includes lymphoma

involving the eyelids, conjunctiva, lacrimal sac, lacrimal gland, orbit, or intraocular space. Ophthalmic lymphoma accounts for approximately 7% of primary extranodal lymphoma cases.

— *Gastrointestinal tract.* This is the most common form of primary extranodal lymphoma, accounting for approximately one-third of cases. The most common site of involvement is the stomach, followed by the ileum, cecum, colon, and rectum. The liver, pancreas, and gallbladder may also be the site of extranodal lymphoma. Symptoms are related to the site involved (eg, nausea, vomiting, diarrhea, bleeding).

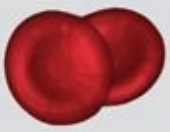
— *Genitourinary tract.* Primary lymphoma of the testes presents as painless enlargement and may be bilateral. Primary lymphoma of the ovary is often bilateral and presents as abdominal masses sometimes felt on abdominal or pelvic examination. Cases limited to the uterus, uterine cervix, vagina, or vulva may occur. Lymphomatous involvement of both kidneys usually presents with renal failure. Bilateral ureteral involvement presents with obstructive renal failure. Primary lymphoma of the bladder or of the prostate may occur.

— *Heart.* Primary cardiac lymphoma may involve the heart or pericardium. Patients may present with dyspnea, edema, arrhythmia, or pericardial effusion with tamponade. Masses may occur in the right atrium (most common), pericardium, right ventricle, left atrium, or left ventricle.

— *Paranasal sinuses.* Lymphoma may present with local pain, upper airway obstruction, rhinorrhea, facial swelling, or epistaxis. It is usually diffuse large B-cell lymphoma in the United States and western Europe and T-cell and NK cell lymphoma in Asia.

— *Skin.* The three main types of cutaneous B-cell lymphoma are primary cutaneous marginal zone B-cell lymphoma, primary cutaneous follicular center B-cell lymphoma, and primary cutaneous large B-cell lymphoma (leg type). The first two are indolent lymphomas and the last type is an aggressive lymphoma. These lymphomas may present as soft tissue masses, mimicking sarcoma, until a biopsy and histopathologic diagnosis is obtained.

— *Spleen.* Primary splenic lymphoma is rare because concomitant marrow involvement is present in most cases. The issue of whether splenic lymphoma is extranodal arises but because lymphoma is usually confined to the red pulp and not the white pulp, it can be considered extranodal.



For a more detailed discussion, see Oliver W. Press and Marshall A. Lichtman: General Considerations of Lymphoma: Epidemiology, Etiology, Heterogeneity, and Primary Extranodal Disease, Chap. 95 in *Williams Hematology,* 9th ed.

CHAPTER 59
Hodgkin Lymphoma

DEFINITION

- The neoplasm of lymphoid tissue in most cases is derived from germinal center B cells, defined by the presence of the Reed-Sternberg cells or its mononuclear variant Hodgkin cells with a characteristic immunophenotype and appropriate cellular background.
- The Reed-Sternberg and Hodgkin cell, the neoplastic cells defining Hodgkin disease, are considered of B-cell origin based on their clonal immunoglobulin gene rearrangements.
- Classic Hodgkin disease accounts for 95% of cases and contains four histologic subtypes that are distinguished on the basis of microscopic appearance and relative proportions of Reed-Sternberg cells, lymphocytes, and fibrosis: nodular sclerosis, mixed cellularity, lymphocyte-depleted, or lymphocyte-rich Hodgkin disease. A fifth subtype, nodular lymphocyte predominance has been added to the four classic histologic types (**Table 59–1**).

TABLE 59–1 CLASSIFICATION OF HODGKIN LYMPHOMA

Histologic Subtype	Immunophenotype
Nodular lymphocyte-predominant	CD20+ CD30– CD15– Ig+
Classic Nodular sclerosis Mixed cellularity Lymphocyte-rich Lymphocyte-depleted	CD20–*CD30+ CD15+ Ig–

Ig, immunoglobin.
Source: *Williams Hematology,* 9th ed, Chap. 97, Table 97–1.

EPIDEMIOLOGY

- In 2014 in the United Stated, there were 9190 cases of Hodgkin lymphoma.
- Incidence rate is influenced by socioeconomic and environmental factors.
- There is a bimodal age distribution, with a peak between ages 15 to 34 and in those older than age 60 years (**Figure 59–1**).
- Nodular sclerosis subtype predominates in young adults.
- Mixed cellularity subtype predominates in older ages.
- Presence of the Epstein-Barr virus (EBV) in Reed-Sternberg and Hodgkin cells is more common in less-developed countries and in pediatric and older adult cases.
- Role for EBV in etiology is suggested by evidence that serologically confirmed mononucleosis confers a threefold risk for Hodgkin disease in young adults.

- Increased risk among siblings and close relatives suggests genetic factors may contribute to disease susceptibility.

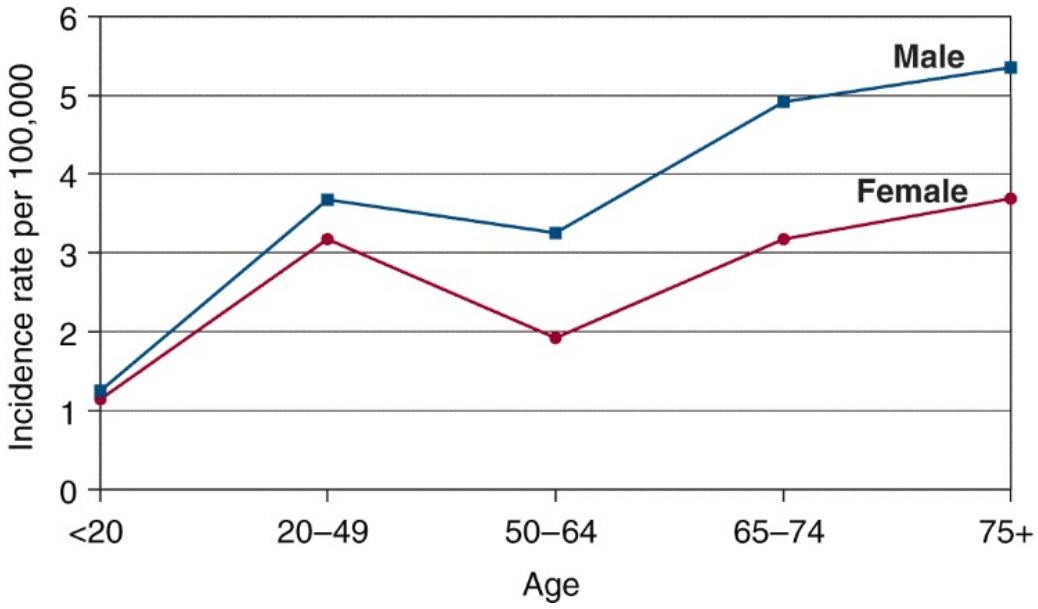


FIGURE 59–1 The graph depicts the incidence of Hodgkin lymphoma as a function of age among American males and females, 2000 to 2011. (Data from the Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) Research Data (1973–2011), National Cancer Institute, DCCPS, Surveillance Research Program, Surveillance Systems Branch, released April 2014, based on the November 2013 submission; 2014. Source: *Williams Hematology*, 9th ed, Chap. 97, Fig. 97–1.)

ETIOLOGY AND PATHOGENESIS

- Reed-Sternberg cells are relatively large cells that typically have bilobed nuclei with prominent eosinophilic nucleoli separated by a clear space from a thickened nuclear membrane (**Figure 59–2**).
- Reed-Sternberg cells express CD30 in virtually all and CD15 in the majority of cases of classic Hodgkin lymphoma (**Figure 59–3**).
- Reed-Sternberg cells represent monoclonal outgrowths of germinal center B cells that have incurred extensive somatic mutations, most likely in the course of the immune response to antigen.
- Mononuclear Reed-Sternberg cell variants, referred to as Hodgkin cells, have similar nuclear characteristics and may represent Reed-Sternberg cells cut in a plane that shows only one lobe of the nucleus.
- Nearly all Hodgkin and Reed-Sternberg cells have rearranged and somatically mutated immunoglobulin VH genes (IgHV).
- It is possible that Hodgkin and Reed-Sternberg cells originate from a preapoptotic germinal center B cell with unfavorable mutations that has escaped negative selection.
- Karyotypes are usually hyperdiploid with structural abnormalities but without pathognomonic chromosomal aberrations.
- Reed-Sternberg cells secrete a variety of cytokines and chemokines that may be responsible for the recruitment of nonmalignant cells that comprise the bulk of the cells in the tumor population.
- Hodgkin and Reed-Sternberg cells show a global loss of their B-cell phenotype, retaining only

B-cell features associated with their interaction with T cells and their antigen-presenting function.

- The lack of expression of numerous B-cell genes is the result of loss of B-cell transcription factor expression (OCT2, BOB1, and PU.1) and epigenetic silencing.
- The main B-cell lineage commitment factor, PAX5, is typically expressed, but its target genes are downregulated.
- Because Hodgkin and Reed-Sternberg cells lack expression of functional B-cell surface receptors, rescue from apoptosis is probably an important mechanism of survival.
- Most prevalent genetic lesions in Hodgkin and Reed-Sternberg cells involve two signaling pathways:
  — Janus kinase (JAK)-STAT. Gains in JAK2 and inactivation of the negative regulator JAK-STAT signaling, suppressor of cytokine signaling 1, result in enhanced cytokine signaling.
  — Nuclear factor-κB (NF-κB). Genetic alterations include gains and amplifications of the NF-κB transcription factor REL in about half of all cases. Somatic mutations of the gene encoding the inhibitor of NF-κB (1κBα) occur in approximately 20% of cases. Inactivating mutations and deletions of the gene encoding A20, a negative regulator of NF-κB, have been found in approximately 40% of cases, nearly all of which were EBV-negative.
- Multiple-receptor tyrosine kinases are aberrantly expressed in Hodgkin and Reed-Sternberg, including platelet-derived growth factor receptor-α.
- Several factors point to the pathogenetic role of EBV in approximately 40% of classic Hodgkin lymphoma.
  — The viral proteins latent membrane protein 1 (LMP1) and latent membrane protein 2 (LMP2), in particular, appear to have hijacked signaling pathways to promote the survival of EBV-infected Hodgkin and Reed-Sternberg cells.
  — There is an inverse relationship between expression of multiple receptor tyrosine kinases and EBV expression.
  — There is an ability of EBV to rescue crippled germinal center B cells in the laboratory.
  — Mutations preventing any B-cell receptor expression are in EBV-positive Hodgkin and Reed-Sternberg cells.
  — There is an inverse relationship between mutations reducing the expression of the NF-κB regulator A20 and EBV-positive Hodgkin and Reed-Sternberg cells.
  — The survival of the malignant cells seems to be dependent on the microenvironment, which represents 95% to 99% of the cellular composition of the tumor.

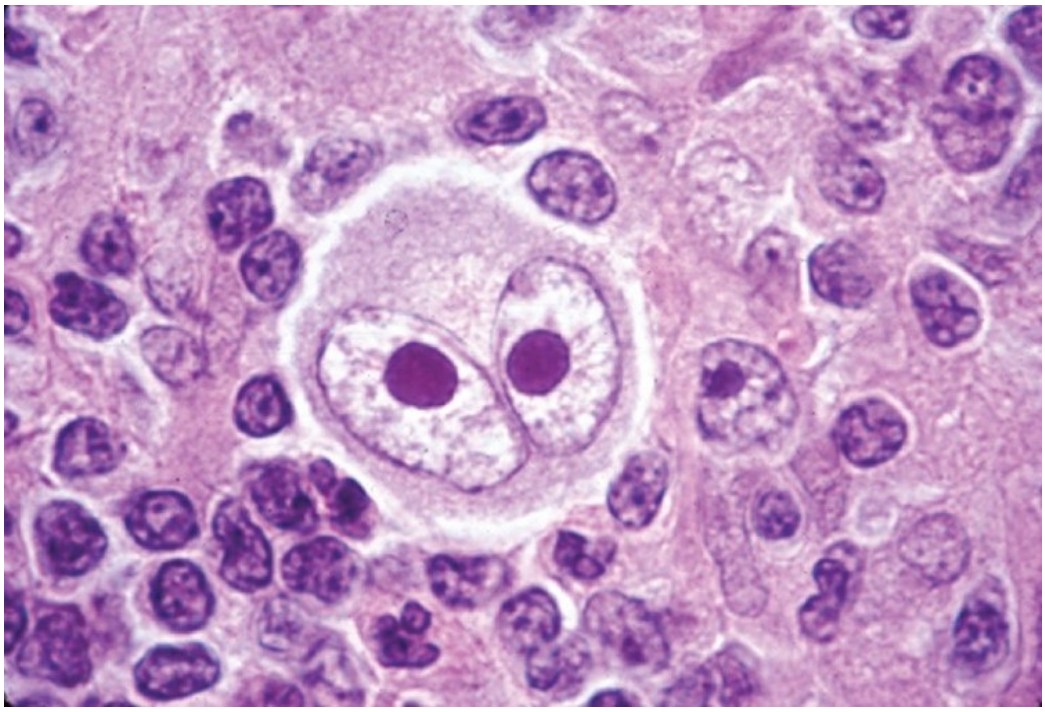


FIGURE 59–2 High magnification of lymph node section in a patient with Hodgkin lymphoma. A Reed-Sternberg cell is in the center of the field with the classical findings of giant size compared to background lymphocytes, binucleation, and prominent eosinophilic nucleoli. (Source: *Williams Hematology*, 9th ed, Chap. 97, Fig. 97–3.)

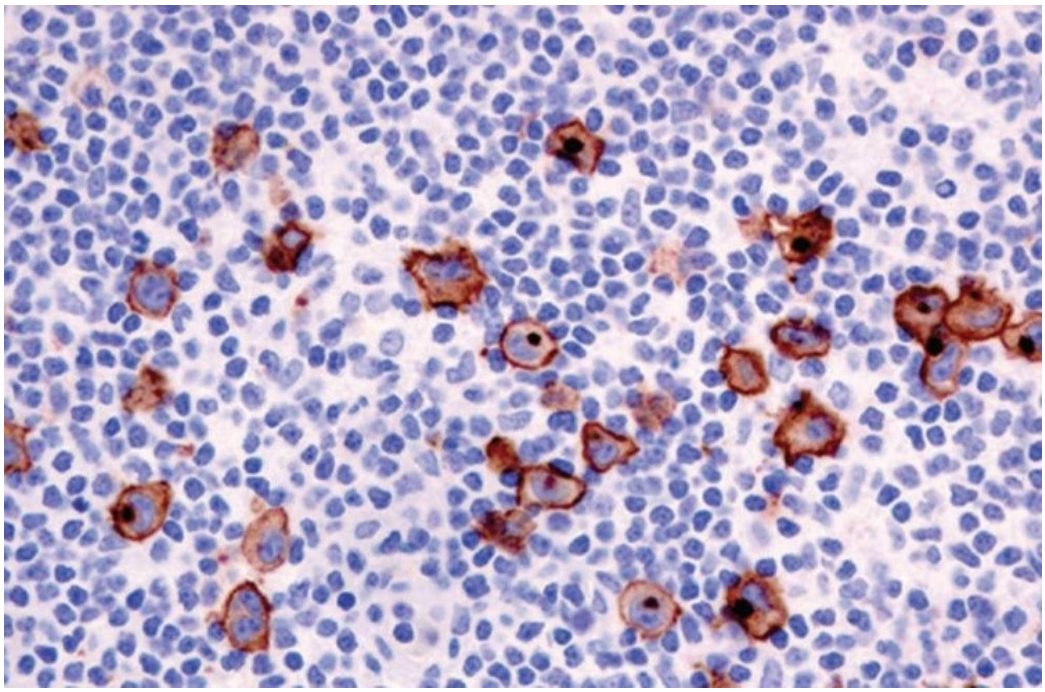


FIGURE 59–3 Classical Hodgkin lymphoma stained with antibody to CD30. CD30 stains the Reed-Sternberg cells in this lymph node biopsy. (Source: *Williams Hematology*, 9th ed, Chap. 96, Fig. 96–36.)

CLINICAL FEATURES

- Usual presentation is with painless lymph node enlargement.
- Constitutional symptoms may be present:
  — "B" symptoms: fever above 38°C, drenching night sweats, and weight loss of more than 10% of baseline body weight

— Pel-Ebstein fever: high fevers for 1 to 2 weeks alternating with afebrile periods of approximately 1 week (virtually diagnostic of Hodgkin disease)

- Detection of an unusual mass or swelling in the superficial, supradiaphragmatic lymph nodes (60%–70% cervical and supraclavicular, 15%–20% axillary) is the most common presentation.
- Pruritus may be evident and pain at the site of involved nodes with alcohol ingestion is an unusual but characteristic finding.
- Intrathoracic disease is present at diagnosis in 67% of patients.
- Mediastinal adenopathy is common.
- Immunologic dysfunction:
  — All patients have multiple abnormalities of cellular immunity.
  — Some defects persist even after successful treatment.
- Abnormalities in blood counts are variable and nondiagnostic.
- A number of rare paraneoplastic syndromes have been described in Hodgkin lymphoma at the time of diagnosis.
  — These include vanishing bile duct syndrome and idiopathic cholangitis with clinical jaundice, the nephrotic syndrome with anasarca, autoimmune hematologic disorders (eg, immune thrombocytopenia or immune hemolytic anemia), and neurologic signs and symptoms.

RADIOGRAPHIC FEATURES

- Whole-body F-F-fluorodeoxyglucose positron emission tomography (FDG-PET) has become the standard imaging study for staging and restaging.

CLINICAL AND PATHOLOGIC CORRELATION

The five histologic subtypes of Hodgkin disease are nodular sclerosis, mixed cellularity, lymphocyte depletion, lymphocyte rich, and nodular lymphocyte predominance. (See **Table 59–1**.)

Nodular Sclerosis

- Approximately 50% of patients present with nodular sclerosis subtype.
- Frequent involvement of the lower cervical, supraclavicular, and mediastinal lymph nodes in adolescents and young adults, particularly females.
  — In young females, there is frequent involvement in lower cervical, supraclavicular, and mediastinal lymph nodes.
  — Seventy percent have limited stage disease.
- Main distinguishing feature is the *lacunar cell*, a Reed-Sternberg cell variant with immunophenotype CD30+ CD15+ CD20– that derives its appearance from retraction of its cytoplasm.

Mixed Cellularity

- This subtype affects both pediatric and older age groups.
- It has a strong association with prior EBV infection.
- It is associated with advance stage disease.
- Mixed cellularity occurs in approximately 40% of patients.
- Classic Reed-Sternberg cells exist amid a cellular background of lymphocytes, eosinophils, plasma cells, and histiocytes.

Lymphocyte-Depletion

- Hodgkin and Reed-Sternberg cells are sparse.
- This subtype is present in older age groups.
- Systemic symptoms are frequent.
- There is widespread disease involving superficial and mediastinal lymph node groups and hepatosplenomegaly.
- Presenting features include fever, jaundice, and pancytopenia.
- This subtype is associated with acquired immunodeficiency disorder (see Chap. 51).

Lymphocyte Rich

- Presenting features are very similar to the nodular lymphocyte-predominant subtype, although patients with the lymphocyte-rich subtype tend to be older.
- Classic CD30+ CD20
  — Immunophenotype of Reed-Sternberg cells.

Nodular Lymphocyte Predominance

- This histologic pattern is found in approximately 5% of patients.
- Patients typically present with disease in a localized lymph node area, especially the axilla.
- Male predominance is 4:1.
- The tumor is composed principally of benign lymphocytes with some histiocytes. Neutrophils and eosinophils are rare.
- CD20+CD15–CD30– lymphocytes with mononuclear but lobulated or folded nucleus referred to as popcorn cells. Unlike classic Hodgkin lymphoma, cells express B-lineage markers (eg, CD20).

ANATOMIC DISTRIBUTION OF HODGKIN DISEASE

- Hodgkin lymphoma presents:
  — In the cervical nodes in 70% of patients
  — In the axillary nodes in 12% of patients
  — In the inguinal nodes in 9% of patients
- The frequency of splenic involvement is approximately 35%.
  — Splenic involvement depends on histologic subtype; it is more frequent in mixed cellularity and lymphocyte depletion cases compared with lymphocyte predominant or nodular sclerosis.

- Disease distribution at presentation:
  — A minority of patients have exclusive subdiaphragmatic disease.
  — FDG-PET provides sensitive delineation of involved sites.
- Staging (**Table 59–2**).
- Use of chemotherapy in all stages of disease has reduced the importance of detecting subclinical disease.
- Marrow involvement occurs in approximately 10% to 15% of new patients and is more common in patients of older age, advanced stage, or less favorable histology. The need for routine bone marrow biopsy in staging is no longer universally accepted.

TABLE 59–2 ANN ARBOR STAGING CLASSIFICATION OF HODGKIN LYMPHOMA

Stage	
I	Involvement of a single lymph node region (I) or of a single extralymphatic organ or site (I_E)
II	Involvement of two or more lymph node regions on the same side of the diaphragm alone (II), or with involvement limited, contiguous extralymphatic organ tissue (II_E)
III	Involvement of lymph node regions on both sides of the diaphragm (III), which may include the spleen (III_S), or limited, contiguous extralymphatic organ, or site (III_E), or both (III_{SE})
IV	Multiple or disseminated foci of involvement of one or more extralymphatic organs or tissues, with or without associated lymph node involvement
Modifying Features	
A	Asymptomatic
B	Drenching night sweats; fever > 38 °C; loss of more than 10% body weight in 6 months
C	Involvement of a single, contiguous or proximal extranodal site

Bulky disease is defined as a mass > 10 cm or a mediastinal mass ratio > 0.33. The mediastinal mass ratio is the ratio of the maximal width of a mediastinal mass relative to the maximum width of the mediastinum, as measured by CT imaging.
Source: *Williams Hematology,* 9th ed, Chap. 97, Table 97–2.

LABORATORY FEATURES

- There are no diagnostic laboratory features of Hodgkin lymphoma.
- A complete blood count may reveal one or another of neutrophilia, eosinophilia, lymphocytopenia, thrombocytosis, or anemia.
- The erythrocyte sedimentation rate (ESR) is prognostic and can be used to follow response.
- Immune neutropenia can occur.
- Anemia: usually a result of chronic disease, but rarely may be caused by hemolysis secondary to high fever or associated with a positive direct antiglobulin (Coombs) test.
- Thrombocytopenia may occur as a result of marrow involvement, hypersplenism, or an immune mechanism (immune thrombocytopenic purpura).
- Serum lactate dehydrogenase levels are elevated in 35% of patients at diagnosis.
- Elevated serum levels of β_2-microglobulin in the setting of normal renal function correlates with tumor burden and prognosis.
- Elevated serum levels of cytokines, including interleukins IL-6 and IL-10, and soluble CD30 and CD2 may correlate with constitutional symptoms and/or advanced disease.
- Hypercalcemia is unusual in Hodgkin lymphoma and appears to be secondary to synthesis of increased levels of 1, 25-dihydroxycalcitriol (vitamin D_3) by Hodgkin lymphoma cells.

DIFFERENTIAL DIAGNOSIS

- Biopsy of unexplained, persistent, or recurrent adenopathy should be reviewed by an experienced hematopathologist.
  — The most likely diagnosis is either Hodgkin or a non-Hodgkin lymphoma.
- Non-neoplastic conditions that simulate Hodgkin lymphoma include viral infections, particularly infectious mononucleosis.
- Cell-depleted nodes of any histology may resemble the diffuse fibrosis variant of lymphocyte-depleted Hodgkin lymphoma, including the depleted phase of lymph nodes from HIV-infected patients.
- With a mediastinal presentation, the distinction between Hodgkin lymphoma and mediastinal large B-cell lymphoma or gray zone lymphoma can be difficult.

TREATMENT

- Favorable, limited-stage disease (**Table 59–3**)
  — All patients initially receive chemotherapy—usually **A**driamycin, **b**leomycin, **v**inblastine, **d**acarbazine (ABVD) for 2 or 4 to 6 cycles.
  — In very favorable patients, ABVD × 2 plus 20 Gy involved field radiotherapy is adequate.
  — There is increasing interest in using ABVD alone to reduce late effects of therapy. For patients who achieve a PET-negative complete remission (CR) no survival disadvantage has been found to withholding radiotherapy, but there appears to be a higher relapse rate.
  — There are several studies ongoing studying the use of interim or post-treatment PET scans to determine the number of cycles of chemotherapy, possible changing chemotherapy regimens, and the possibility of avoiding radiotherapy.
- Unfavorable limited-stage disease (see **Table 59–3**)
  — Four cycles of ABVD plus 30 Gy involved field radiotherapy are adequate.
  — PET-directed therapy is being studied, but eliminating radiotherapy yielded a significantly higher relapse rate.
- Advanced disease
  — ABVD and escalated **b**leomycin, **e**toposide, **A**driamycin, **c**yclophosphamide, vincristine (**O**ncovin), **p**rocarbazine, **p**rednisone (BEACOPP) are the most widely used regimens.
  — Escalated BEACOPP has a significantly lower relapse rate, but when autologous bone marrow transplants are utilized in relapsing patients there has not been a difference in overall survival.
  — In patients who are PET-negative after completing chemotherapy, there appears to be no advantage to adjuvant radiotherapy.
  — Interim PET scans are being studied to see if modifying therapy based on the result can improve outcome.
  — ABVD with the anti-CD 30 antibody drug conjugate brentuximab vedotin substituted for bleomycin (ie, pulmonary toxicity precluded the use of both drugs) is being studied with impressive early results.
- Regimens for combination chemotherapy are presented in **Table 59–4**.

TABLE 59–3 PROGNOSTIC FACTORS FOR HODGKIN LYMPHOMA

Limited Stage		Advanced Stage
EORTC	**GHSG**	**International Collaborative Study**
Adverse Prognostic Factors		**Adverse Prognostic Factors**
MMR ≥ 0.35	MMR ≥ 0.35	Age ≥ 45 years
ESR > 30 if symptomatic	ESR > 30 if asymptomatic	Stage IV
ESR > 50 if asymptomatic	ESR > 50 if asymptomatic	Male sex
> 3 Ann Arbor sites	> 2 Ann Arbor sites	White blood count ≥ 15×10^9/L
Age ≥ 50	Extranodal disease	Lymphocyte count < 0.6×10^9/L or < 8%
	Massive splenic disease	Albumin < 4 g/dL
Presence of any factor is considered unfavorable.		Hemoglobin < 10.5 g/dL
Two-thirds of limited stage patients have one or more adverse factors.		Factors summed to yield the international prognostic score 75% of patients have a score of 1–3

EORTC, European Organization for the Research and Treatment of Cancer; ESR, erythrocyte sedimentation rate; GHSG, German Hodgkin Study Group; MMR, mediastinal mass ratio, which is the ratio of the maximal width of a mediastinal mass relative to the maximal width of the mediastinum, as measured by CT imaging.
Source: *Williams Hematology*, 9th ed, Chap. 97, Table 97–4.

TABLE 59–4 COMBINATION CHEMOTHERAPY FOR HODGKIN LYMPHOMA

Drug	Dose (mg/m^2)	Route	Schedule (Days Administered)	Cycle Length (Days)
ABVD				28
Doxorubicin	25	IV	1, 15	
Bleomycin	10	IV	1, 15	
Vinblastine	6	IV	1, 15	
Dacarbazine	375	IV	1, 15	
COPP				28
Cyclophosphamide	650	IV	1, 8	
Vincristine	1.4*	IV	1, 8	
Procarbazine	100	PO	1–14	
Prednisone	40	PO	1–14	
COPP/ABVD				28
Alternate cycles of COPP with ABVD				
BEACOPP (Standard)				21
Bleomycin	10	IV	8	
Etoposide	100	IV	1–3	
Doxorubicin	25	IV	1	
Cyclophosphamide	650	IV	1	
Vincristine	1.4*	IV	8	
Procarbazine	100	PO	1–7	
Prednisone	40	PO	1–14	

BEACOPP (Escalated)				21
Bleomycin	10	IV	8	
Etoposide	200	IV	1–3	
Doxorubicin	35	IV	1	
Cyclophosphamide	1250	IV	1	
Vincristine	1.4*	IV	8	
Procarbazine	100	PO	1–7	
Prednisone	40	PO	1–14	
G-CSF	(+)	SQ	8+	
BEACOPP (14-day)				14
Standard BEACOPP given every 14 days with growth factor support				
STANFORD V				12 weeks
Nitrogen mustard	6	IV	day 1 on wk 1, 5, 9	
Doxorubicin	25	IV	day 1 on wk 1, 3, 5, 7, 9, 11	
Vinblastine	6	IV	day 1 on wk 1, 3, 5, 7, 9, 11	
Vincristine	1.4*	IV	day 1 on wk 2, 4, 6, 8, 10, 12	
Bleomycin	5	IV	day 1 on wk 2, 4, 6, 8, 10, 12	
Etoposide	60 × 2	IV	day 1 & 2 on wk 3, 7, 11	
Prednisone	40	PO	day 1 on wk 1–10, taper	
G-CSF for dose reduction, delay				

G-CSF, granulocyte colony-stimulating factor; SQ, subcutaneously.
*Capped at 2 mg.
Source: *Williams Hematology*, 9th ed, Chap. 97, Table 97–3.

Recurrent Disease

- Chemotherapy following relapse after radiotherapy results in an excellent rate of cure.
- High-dose therapy and autologous marrow or peripheral blood stem cell transplantation is the treatment of choice for patients who fail primary induction combination chemotherapy or who relapse after chemotherapy.
- Autologous transplantation cure rates range from 40% to 60%.
- High-dose regimens include BEAM (**c**armustine, **e**toposide, **c**ytarabine, **m**elphalan), CBV (cyclophosphamide, **c**armustine, **e**toposide), and augmented CBV regimens.
- Second-line chemotherapy with ICE (**i**fosfamide, **c**arboplatin, **e**toposide), DHAP (**d**examethasone, **c**ytarabine, **c**isplatin), or IGEV (**i**fosfamide, **g**emcitabine, **v**inorelbine) is used to achieve a minimal disease state prior to stem cell mobilization and transplantation.
- Treatment failures following autologous transplantation present a challenge, with longevity directly related to the time to relapse after transplant.

- The anti-CD30 antibody drug conjugate brentuximab vedotin is the most active agent in relapsed/refractory disease with more than a 90% tumor shrinkage rate and 34% complete remission rate. The major toxicity is neuropathy.
- The human programmed death receptor-1 blockers nivolumab and pembrolizumab are very promising new agents with novolumab achieving an 86% objective response rate in heavily pretreated patients.

COURSE AND PROGNOSIS

- Survival rate is 90% at 10 years up to age 44 years, 80% at 10 years up to 54 years, and 70% at 10 years up to 64 years of age.

Clinical Prognostic Markers

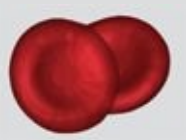
- Prognostic markers for Hodgkin lymphoma are listed in **Table 59–3**.
- Adverse prognostic factors:
  — Male sex
  — Age ≥ 45 years
  — Stage IV disease
  — White blood count greater than or equal to 15×10^9/L
  — Lymphocyte count less than 0.6×10^9/L or less than 8% of total leukocytes
  — Hemoglobin concentration less than 10.5 g/dL
  — Albumin concentration less than 4 g/dL
- The European Organization for the Research and Treatment of Cancer (EORTC) defines four or more nodal sites, ESR greater than 50 mm/h in asymptomatic patients or greater than 30 mm/h in symptomatic patients, and histology as indicators of intermediate disease.
- The presence of each factor reduced freedom from progression by about 7%.
- Patients in the worst prognostic group (five to seven factors) had a 42% freedom from progression at 5 years.
- FDG-PET imaging at the completion of treatment provides a high degree of negative predictive value, ranging from 81% to 100%.
- The positive predictive value of PET scanning at the end of chemotherapy is more variable and is related to disease extent and use of radiotherapy.
- Among patients referred for transplantation, sensitivity to standard-dose, second-line chemotherapy predicts for better survival; responding patients had an event-free survival of 60% versus 19% for those without a response.

Complications of Treatment

- The treatment of Hodgkin lymphoma is associated with important acute and chronic side effects.
- Late-treatment effects in the form of second malignancy and cardiopulmonary disease can contribute to shortened longevity for cured patients. They increase with time and are currently the leading causes of death for Hodgkin lymphoma patients.
- Secondary malignancies:

— Acute leukemia or myelodysplasia. The risk is proportional to cumulative dose of alkylating agents.
— Increased risk of non-Hodgkin lymphoma. Usually diffuse, aggressive, and B cell, and it is more frequent in nodular lymphocyte-predominant subtype.
— Increased risk of development of solid cancer. Cancer is most often involves breast, lung, stomach, bone, or soft tissue, primarily related to site of radiotherapy.

- Cardiac disease may occur in recipients of mediastinal irradiation.
- Radiation pneumonitis may occur depending on dose received by lung.
- Thyroid function abnormalities are common after neck irradiation, reaching a risk of 47% at 26 years.
- Current therapy programs use low-dose or no radiotherapy for all stages of disease.



For a more detailed discussion, see Oliver W. Press: Hodgkin Lymphoma, Chap. 97 in *Williams Hematology*, 9th ed.

CHAPTER 60
Diffuse Large B-Cell Lymphoma and Related Diseases

DEFINITION

- Diffuse large B-cell lymphomas (DLBCLs) are a heterogeneous group of aggressive lymphomas of large, transformed B cells.
- DLBCLs can arise de novo or may transform from a low-grade lymphoma, such as small lymphocytic lymphoma or follicular lymphoma.
- **Table 60–1** lists the variants and subtypes of DLBCL.

TABLE 60–1 DIFFUSE LARGE B-CELL LYMPHOMA: VARIANTS AND SUBTYPES

I. Diffuse large B-cell lymphoma, not otherwise specified (NOS)
- A. Common morphologic variants
  - 1. Centroblastic
  - 2. Immunoblastic
  - 3. Anaplastic
- B. Rare morphologic variants
- C. Molecular subgroups
  - 1. Germinal center B-cell–like
  - 2. Activated B-cell–like
- D. Immunohistochemical subgroups
  - 1. CD5-positive DLBCL
  - 2. Germinal center B-cell–like
  - 3. Nongerminal center B-cell–like

II. Diffuse large B-cell lymphoma subtypes
- A. T-cell/histiocyte-rich large B-cell lymphoma
- B. DLBCL associated with chronic inflammation
- C. EBV-positive DLBCL of the elderly*

III. Related mature B-cell neoplasms
- A. Primary mediastinal (thymic) large B-cell lymphoma
- B. Intravascular large B-cell lymphoma
- C. Primary cutaneous DBCL, leg type*
- D. Lymphomatoid granulomatosis
- E. ALK-positive DLBCL
- F. Plasmablastic lymphoma
- G. Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease
- H. Primary effusion lymphoma

IV. Borderline cases
- A. B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma*
- B. B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classic Hodgkin lymphoma

ALK, anaplastic lymphoma kinase; DLBCL, diffuse large B-cell lymphoma; EBV, Epstein-Barr virus; HHV, human herpes virus; NOS, not otherwise specified.
*These represent provisional entities or provisional subtypes of other neoplasms.

Source: *Williams Hematology,* 9th ed, Chap. 98, Table 98–1.

EPIDEMIOLOGY

- This is the most common B-cell lymphoid neoplasm in the United States and Europe and accounts for approximately 28% of all mature B-cell lymphomas.
- The most common presentation is in late middle-aged and older persons.
- Median age at diagnosis is approximately 65 years.

ETIOLOGY AND PATHOGENESIS

- This molecularly heterogeneous disease with multiple complex chromosomal translocations and genetic abnormalities is identified by cytogenetics and gene expression profiling.
- Disease is derived from B cells that have undergone somatic mutation in the immunoglobulin (Ig) genes.
- *BCL6* gene rearrangements may be specific for DLBCL.
  - — Approximately 40% of cases in immunocompetent persons and approximately 20% of human immunodeficiency virus (HIV)-related cases display *BCL6* rearrangements.
  - — BCL6 protein mediates the specific binding of several transcription factors to DNA.
- Approximately 30% of patients have the t(14;18) translocation involving *BCL2* and the Ig-heavy-chain gene.
  - — The presence of *p53* mutation in combination with *BCL2* denotes that the tumor is derived from a transformation of a prior follicular lymphoma.
- Aberrant somatic mutation occurs in more than 50% of cases and targets multiple loci (eg, *IGH, PIM1, MYC, RhoH/TTF* [ARHH], *PAX5, c-MYC*).
- Three molecular subtypes have been identified determined by gene expression profiling:
  - — Germinal center B-like (GCB) arise from normal germinal center B cells (**Table 60–2**).
  - — Activated B-cell–like (ABC) may arise from postgerminal center B cells that are arrested during plasmacytic differentiation (see **Table 60–2**).
  - — Primary mediastinal B-cell lymphoma might arise from thymic B cells.

TABLE 60–2 **DIFFUSE LARGE B-CELL LYMPHOMA SUBTYPES ARE DISTINGUISHED BY DISTINCT MUTATIONS IN THE CELLS OF ORIGIN**

	GCB DLBCL			ABC DLBCL	
Mutation	**Frequency**	**Effect**	**Mutation**	**Frequency**	**Effect**
BCL2 translocation	25%	Antiapoptotic	PRDM1	50%	Differentiation block
EZH2 mutations	22%	Histone modification	A20 loss	20%	NF-κB activation
MEF2B mutations	22%	Chromatin remodeling	CD79B mutations	21%	NF-κB/BCR signaling
MYC translocation	5%	Proliferation	CARD11 mutations	11%	NF-κB activation
TNFRSF14 mutations	13%	Immune escape	MYD88 mutations	29%	NF-κB/JAK-STAT signaling
GNA 12 and 13 mutations	29%	GTPases; B-cell homing			

ABC, activated B-cell–like; BCR, breakpoint cluster region; DLBCL, diffuse large B-cell lymphoma; GCB, germinal center B-cell–like; GTPase, guanosine triphosphatase; JAK, Janus kinase; NF-κB, nuclear factor kappaB; STAT, signal transducer and activator of transcription.

Source: *Williams Hematology*, 9th ed, Chap. 98, Table 98–2.

CLINICAL FEATURES

- Lymph nodes are enlarged, nontender, and firm but rubbery, and they are typically found in the neck or as a mass in the abdomen.
- Systemic "B" symptoms of fever, night sweats, and weight loss occur in 30% of patients at presentation.
- Approximately 60% of patients have disseminated DLBCL (stage III or IV) on presentation.
- Other extranodal sites that may be affected include testis, bone, thyroid, salivary glands, skin, liver, breast, nasal cavity, paranasal sinuses, pleural cavity, and central nervous system (CNS).
- Marrow involvement occurs in 15% of patients.
- CNS involvement may occur in concert with testicular or paranasal sinuses involvement.
- Some patients might have discordant disease in which the lymph nodes are involved with DLBCL but the marrow histopathology may be that of a low-grade lymphoma.
- Patients with lymphoma in the Waldeyer ring have an increased risk of gastrointestinal lymphoma.

LABORATORY FEATURES

Blood and Marrow

- Lymphoma cells in blood are found in approximately 5% of cases.
- Lymphoma involvement of the marrow is found in approximately 15% of cases.

Cell Immunophenotype

- The malignant cells have surface monoclonal Ig of either κ or λ light-chain type.
  — The most commonly expressed surface Ig is IgM.
- Lymphoma cells generally express the pan-B cell antigens CD19, CD20, CD22, PAX5, and CD79a.
  — The cells also express CD45 and less commonly CD10 or CD5.
- CD5+ DLBCL may be more aggressive with worse prognosis.

Histopathology

- Three cytologic patterns of lymphocytes are recognized: centroblastic, immunoblastic, and anaplastic.
- Lymph nodes are usually effaced by a diffuse infiltrate of large lymphocytes.
- Other rare morphologic variants occur, for example, with a myxoid or fibrillary appearance.

PROGNOSTIC FACTORS

- In 1993, a model was proposed to assign a prognosis to patients with aggressive lymphoma undergoing treatment with doxorubicin-containing chemotherapeutic regimens termed the *international prognostic index* (IPI) (**Table 60–3**).
- The 5-year survival rates for patients age 60 years or younger with IPI scores of 0, 1, 2, and 3 were 83%, 69%, 46%, and 32%, respectively (**Table 60–4**).
- Gene expression profiling has also been used to delineate groups of patients with DLBCL who may differ in their response to therapy and prognosis (**Figure 60–1**).
- The relative expression of six genes can identify three prognostic groups:
  — High-level expression of *LMO2, BCL6,* and *FNI* correlated with prolonged survival
  — High-level expression of *BCL2, CCND2,* and *SCYA3* correlated with short survival
- Patients with an elevated β_2-microglobulin level and high serum lactic acid dehydrogenase (LDH) have a poor prognosis.
- Approximately 70% of DLBCL cases are of germinal center origin, as demonstrated by BCL6 protein and have a more favorable prognosis.
- Survivin, a member of the inhibitor of apoptosis family of proteins, is expressed in 60% of patients with DLBCL and is associated with a poor prognosis.
- High number of infiltrating CD4+ T cells in lymph nodes involved with DLBCL is associated with a better prognosis.
- High-level expression of cyclin D3; serum vascular endothelial growth factor; and plasma cytokines such as interleukin (IL)-2, IL-10, and IL-6, or *p53* gene mutation are associated with a poor prognosis.
- Fluorine-18-fluorodeoxyglucose-positron emission tomography (FDG-PET) is used for staging and monitoring patient's response to therapy.
- A negative PET scan at the end of therapy is the best definition for a complete remission and best predictor of survival free from relapse.

TABLE 60–3 INTERNATIONAL PROGNOSTIC FACTOR INDEX FOR NON-HODGKIN LYMPHOMA

Risk Factors
Age > 60 years
Serum lactic acid dehydrogenase greater than twice normal
Performance status ≥ 2
Stage III or IV
Extranodal involvement at more than one site

Each factor accounts for 1 point, for a total score that ranges from 0 to 3 for patients < 61 years of age. The latter age-adjusted index includes all variables except for age and extranodal sites. For patients ≥ 61 years of age, a total score ranges from 0 to 5 and includes each variable shown in this table.
Source: *Williams Hematology,* 9th ed, Chap. 98, Table 98–5.

TABLE 60–4 OUTCOME ACCORDING TO RISK GROUP DEFINED BY THE INTERNATIONAL PROGNOSTIC INDEX

International Index	No. of Risk Factors	Complete Response Rate (%)	Relapse-Free Survival (%)	Survival (%)

International Prognostic Index, All Patients						
			2-Year	*5-Year*	*2-Year*	*5-Year*
Low	0 or 1	87	79	70	84	73
Low-intermediate	2	67	66	50	66	51
High-intermediate	3	55	59	49	54	43
High	4 or 5	44	58	40	34	26
Age-Adjusted International Index, Patients <61 Years of Age						
			2-Year	*5-Year*	*2-Year*	*5-Year*
Low	0	92	88	86	90	83
Low-intermediate	1	78	74	66	79	69
High-intermediate	2	57	62	53	59	46
High	3	46	61	58	37	32

Source: *Williams Hematology,* 9th ed, Chap. 98, Table 98–6.

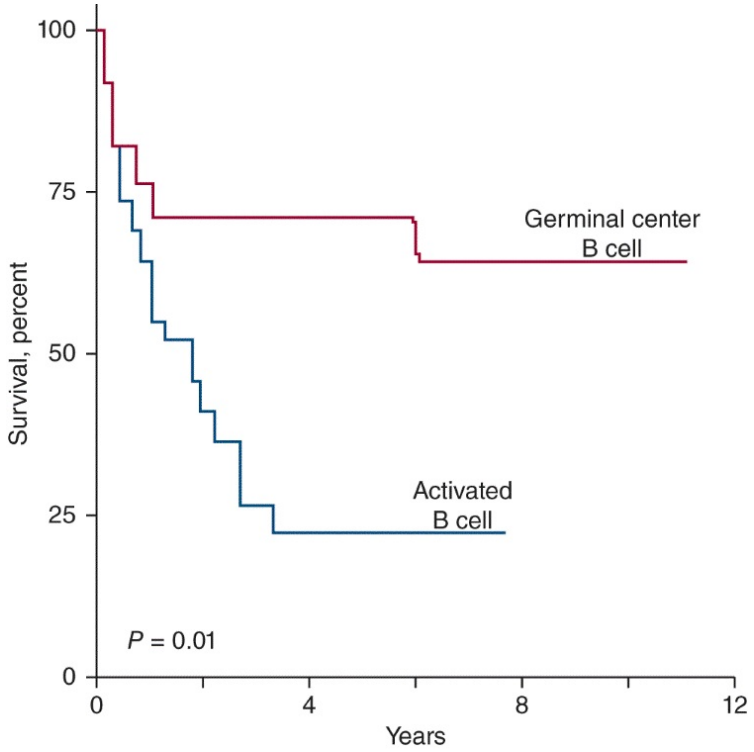


FIGURE 60–1 Overall survival in a group of patients with diffuse large B-cell lymphoma whose cell of origin was determined by gene expression profiling. Survival of patients with diffuse large B-cell lymphoma whose malignant cells were thought to arise from a germinal center B cell was significantly better than in patients whose cell of origin arose from activated B cells. (Source: *Williams Hematology*, 9th ed, Chap. 98, Figure 98–2.)

TREATMENT

- DLBCL is potentially curable with combination chemotherapy.
- The best outcomes are seen when full doses are administered on schedule.

Early Stage DLBCL (Stages I and II)

- Localized disease occurs in approximately 25% of patients.
- Combining an abbreviated course of immunochemotherapy with radiation therapy or giving a complete course of immunochemotherapy and only using radiotherapy in patients with a residual abnormal PET scan is each a reasonable approach.
- Table 60–5 contains treatment approaches for limited-stage aggressive DLBCL.
- Radiotherapy might particularly benefit patients older than age 60 and those with large masses (ie, > 7.5 cm).
- Rituximab has changed the therapeutic paradigm in advanced DLBCL and is incorporated in most treatment regimens.

TABLE 60–5 TREATMENT OF LIMITED-STAGE AGGRESSIVE LYMPHOMA

Patient Population	Number of Patients	Treatment	5-Year OS (*P* value) (%)
Stages I and II, nonbulky	401	Eight cycles CHOP vs three cycles CHOP + IFRT	72 (*P* = .05) 82
Bulky stages I, IE, II, and IIE	399	Eight cycles CHOP vs eight cycles CHOP + IFRT	73* 87 (*P* = .24)
Age > 60 years, IPI O	576	Four cycles CHOP vs four cycles CHOP + IFRT	72 68 (*P* = .5)
Age < 61 years, localized stages I and II, IPI O Age > 60 years with IPI > O	647 60	ACVBP vs four cycles CHOP + IFRT R-CHOP + IFRT	90 87 (*P* < .001) 92

CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; IFRT, involved-field radiation therapy; IPI, international prognostic index; OS, overall survival; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone.
*OS for 172 complete remission patients randomized to observation versus involved-field radiation therapy.
Source: *Williams Hematology,* 9th ed, Chap. 98, Table 98–3.

Advanced Stages of DLBCL (Bulky Stages I and II or III and IV)

- Table 60–6 lists different chemotherapy regimens for intermediate- and high-grade lymphoma.
- **R**ituximab-**c**yclophosphamide, **h**ydroxydaunorubicin (doxorubicin), vincristine (**O**ncovin) and **p**rednisone (R-CHOP) now is the standard of care for younger patients with DLBCL.
  - — Comparing R-CHOP and R-EPOCH (addition of etoposide to R-CHOP) could modify current treatment recommendations.
  - — It may be that certain genetic subgroups will benefit from specific regimens.

TABLE 60–6 COMBINATION CHEMOTHERAPY FOR INTERMEDIATE- AND HIGH-GRADE LYMPHOMA

Regimen	Dose	Route	Days of Treatment	Interval Between Treatment Cycles (Days)	Cycles
R-CHOP-21					

Rituximab	375 mg/m^2	IV	1	21	6–8
Cyclophosphamide	750 mg/m^2	IV	1		
Doxorubicin	50 mg/m^2	IV	1		
Vincristine	1.4 mg/m^2	IV	1		
Prednisone	100 mg/d	PO	1–5		
CHOP-14					
Cyclophosphamide	750 mg/m^2	IV	1	14	6–8
Doxorubicin	50 mg/m^2	IV	1		
Vincristine	1.4 mg/m^2	IV	1		
Prednisone	100 mg/d	PO	1–5		
Dose-adjusted R-EPOCH*					
Rituximab	375 mg/m^2	IV	1	21	6–8
Etoposide	50 mg/m^2/d	CIV	1–4 (96 hours)		
Doxorubicin	10 mg/m^2/d	CIV	1–4 (96 hours)		
Vincristine	0.4 mg/d	CIV	1–4 (96 hours)		
Cyclophosphamide	750 mg/m^2/d	IV	5		
Prednisone	60 mg/m^2/d	PO	1–5		
ESHAP (for relapsed lymphoma)					
Etoposide	40 mg/m^2	IV	1–4	21	
Methylprednisone	500 mg/m^2	IV	1–5		
Cytarabine	2 mg/m^2	IV	5		
Cisplatin	25 mg/m^2	CIV	1–4		
DHAP (for relapsed lymphoma)					
Dexamethasone	40 mg/m^2	PO or IV	1–4	21	
Cisplatin	100 mg/m^2	CIV	1		
Cytarabine	2 gm/m^2	IV q12h × 2 doses	2		
R±ICE (for relapsed lymphoma)					
Rituximab	375 mg/m^2	IV	1	14	
Mesna	5000 mg/m^2	IV	1 (day 2)		
Carboplatin	AUC = 5 (maximum 800 mg)	IV	1 (day 2)		
Etoposide	100 mg/m^2	IV	1–3		
Neulasta	6 mg	SQ	1 (day 4)		

AUC, area under the curve; CIV, continuous intravenous infusion; SQ, subcutaneously.
*Doses of etoposide, doxorubicin, and cyclophosphamide are increased 20% over the dose in the previous cycle if the nadir of the absolute neutrophil count in the previous cycle was ≥ 0.5×10^9/L.
The reader is advised to verify drugs, doses, routes, and administration schedules of these regimens.
Source: *Williams Hematology*, 9th ed, Chap. 98, Table 98–4.

Chemotherapy in Patients Older than Age 60 Years

- Patients older than age 60 years with a low or low-intermediate IPI have a lower relapse-free and overall survival rate than younger patients.
- The best therapy based on recent studies for patients over the age of 60 years is six cycles of R-CHOP.

Role of High-Dose Chemotherapy and Autologous Hematopoietic Stem Cell Transplantation in Initial Therapy

- High-dose chemotherapy and autologous hematopoietic stem cell transplantation (HSCT) is not recommended for most patients with DLBCL.
- A subgroup of patients with poor prognostic features such as having two or three factors in the age adjusted IPI may benefit from such aggressive therapy and should be considered for auto-HSCT.

Recurrent and Refractory DLCBL

Chemotherapy

- A substantial proportion of patients are either refractory or will relapse after chemotherapy.
- Relapse usually occurs within the first 2 to 3 years after diagnosis but can occur later.
- Cure of relapsed or refractory patients may first require response to a differently configured regimen followed by autologous HSCT.
- Responses to monotherapy are generally not long-lasting.
- The addition of rituximab to the ifosfamide-carboplatin-etoposide (ICE) chemotherapy regimen (R-ICE) increased the complete response rate of patients with relapsed or primary refractory DLBCL under consideration for autologous HSCT.

Autologous Stem Cell Transplantation (Auto-HSCT)

- Patients with relapsed or primary refractory DLBCL who achieve complete response before auto-HSCT have better outcomes on average than those who achieve only partial response.
- Disease sensitivity at the time of auto-HSCT is the most significant prognostic variable for predicting treatment outcome.
- Patients who undergo auto-HSCT when the disease is resistant to the initial induction therapy have less than a 20% probability of post-transplant disease-free survival.

Allogeneic Hematopoietic Stem Cell Transplantation (Allo-HSCT)

- The overall relapse and progression rate for the allo-HSCT patients at 5 years was 23% compared with 38% in the auto-HSCT patients.
- Allo-HSCT cannot be recommended before auto-HSCT except in the context of a clinical trial.

Principles of Therapy for Relapsed or Refractory Patients

- Radioimmunotherapy as monotherapy is not recommended for DLBCL.

- Patients with relapsed disease should receive multidrug chemotherapy.
- Auto-HSCT should be performed if chemosensitivity is demonstrated and no contraindications are present.
- If patients are elderly or have comorbid conditions, the goal should be palliation.
- Radiotherapy can be used to alleviate symptoms at a particular site of involvement.

Treatment of Specific Subtypes and Clinical Presentations

Primary Testicular Lymphoma

- This type of lymphoma represents 1% to 2% of all lymphomas, with an estimated incidence of 0.26 per 100,000 males per year.
- It represents the most common testicular tumor in men older than 50 years of age.
- Eighty percent to 90% of primary testicular lymphomas are DLBCL, with a mean age at diagnosis of 68 years.
- Most patients present with stage I–II disease with isolated involvement of the right or left testis equal in frequency.
- Six percent of testicular lymphoma cases have bilateral involvement.
- Primary testicular lymphoma tends to disseminate to several extranodal sites, including the contralateral testis, CNS, skin, Waldeyer ring, lung, pleura, and soft tissues.
- Treatment using radiation therapy alone provides suboptimal disease control, even for patients with stage I disease.
- Chemotherapy with anthracycline-containing regimens (eg, R-CHOP) is recommended after orchiectomy.
- Patients should have radiotherapy to the remaining testicle and CNS prophylaxis should be strongly considered.

Lymphoma during Pregnancy

- This lymphoma is the fourth most frequent malignancy diagnosed during pregnancy, occurring in approximately 1 in 6000 deliveries.
- The risks to the fetus of treatment are greatest during the first trimester.
- Patients with supradiaphragmatic stage I disease may be considered for localized radiotherapy as a temporary measure until the second trimester, when chemotherapy holds less risk for the fetus.
- Patients in the second and third trimesters should be treated with immunochemotherapy.

PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA

- This lymphoma arises in the mediastinal lymphatic structures, probably from a thymic B-cell precursor.
- Variant type of DLBCL accounts for approximately 3% of lymphomas and is most commonly seen in young and middle-aged adults, with about two-thirds of cases in females.

Clinical Features

- The clinical presentation is typically an anterior mediastinal mass that is locally invasive of neighboring tissues that may lead to airway obstruction and superior vena cava syndrome in approximately 40% of patients.
- Distant nodal involvement at presentation is more suggestive of typical DLBCL with mediastinal involvement.
- Relapses tend to be extranodal, including the liver, gastrointestinal tract, kidneys, ovaries, and CNS.
- Marrow involvement is very unusual.

Histopathologic Findings

- Primary mediastinal B-cell lymphoma and Hodgkin lymphoma share gene expression profiles, raising questions about biologic relationships.
- Rarely, multinucleated cells may sometimes mimic Reed-Sternberg cells along with other morphologic similarities to Hodgkin lymphoma.
- Fibrotic bands may intersperse with the tumor cells, sometimes referred to as primary B-cell mediastinal lymphoma with sclerosis.
- Primary mediastinal lymphoma lacks the CD30 and CD15 antigens characteristic of Hodgkin lymphoma, and it expresses the B-cell–associated antigens CD19, CD20, CD22, and CD79a.

Treatment

- Incorporation of rituximab in dose-adjusted EPOCH with no radiotherapy resulted in a 91% event-free survival.
  — R-CHOP, often followed by radiotherapy, is also an effective regimen.

LYMPHOMATOID GRANULOMATOSIS

- This rare lymphoproliferative disorder is characterized by angiocentric and angiodestructive Epstein-Barr virus (EBV)-positive B-cell proliferation associated with extensive reactive T-cell infiltration.
  — Approximately two-thirds of cases occur in males.
  — The median age of presentation is in the fifth decade of life, although pediatric cases occur.

Clinical Findings

- The most common site of involvement is the lungs (90%). Other common sites of involvement include the skin (~40%), kidney (~35%), liver (~30%), and the CNS (~25%).
- The spleen and lymph nodes are often less involved.
- The distribution of disease leads to cough, dyspnea, and sometimes chest pain.
- Fever, weight loss, and joint pain are very frequent.
- Abdominal pain and diarrhea as a result of gastrointestinal involvement and various neurologic signs, including diplopia, ataxia, mental status changes, and others may be evident.
- Skin involvement can be morphologically diverse (eg, ulcerations, plaques, maculopapules) but is usually accompanied by subcutaneous nodules.
- The pulmonary lesions are usually bilateral nodules in the lower half of the lung. They may

cavitate. Nodules may also be found in the brain and kidney and sometimes other locales.

Histopathologic Findings

- The grade of lymphomatoid granulomatosis is determined by the proportion of EBV-positive B cells relative to the reactive lymphocytes in the background.
- Grade 1 lesions contain a polymorphous lymphoid infiltrate without cytologic atypia.
- Grade 2 lesions contain occasional large lymphoid cells or immunoblasts in a polymorphous background.
- Grade 3 lesions contain frequent large atypical cells.

Treatment and Prognosis

- The clinical prognosis is variable in lymphomatoid granulomatosis with a median survival of 2 years.
- Poor prognostic findings include neurologic involvement and higher pathologic grade.
- Treatment consists of interferon in grade 1 and 2 lesions and immunochemotherapy as is used in diffuse large B-cell lymphoma for grade 3 lesions.

INTRAVASCULAR LARGE B-CELL LYMPHOMA

- This rare type of extranodal large B-cell lymphoma is characterized by selective growth of lymphoma cells within the lumen of vessels, sparing the large arteries and veins.
- This tumor usually occurs in adults in the sixth and seventh decade.
- It occurs equally in men and women.
- The clinical manifestations of this lymphoma are extremely variable.
- Symptoms are related to the organs affected.
- Two types of clinical patterns have been recognized:
  - — In European countries, patients develop brain and skin involvement.
  - — In Asian countries, patients present with multiorgan failure, hepatosplenomegaly, pancytopenia, and hemophagocytic syndrome.
  - — B symptoms (fever, drenching night sweats, and weight loss) are common in both types.
- A cutaneous variant is seen in females in Western countries.
  - — The lesions may be painful and appear as violaceous plaques, erythematous nodules, or tumors that may ulcerate.
  - — These lesions commonly appear on the arms and legs, abdomen, and breasts but may occur anywhere.
- Increased LDH levels and β_2-microglobulin levels are observed in the serum of most patients.
- Elevated erythrocyte sedimentation rate and abnormalities in hepatic, renal, and thyroid function are common.
- Tumor cells express B-cell–associated antigens and occasionally express CD5.
- Rituximab/anthracycline-based chemotherapy has been used for treatment.
  - — Progression-free survival and overall survival rates at 2 years after diagnosis were 56% and 66%.
- For patients with CNS involvement, more intensive chemotherapy with drugs such as

methotrexate and cytarabine that reach the CNS is required.

POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS

- Post-transplant lymphoproliferative disorder (PTLD) results from lymphoid or plasmacytic proliferations that develop in the setting of immunosuppressive therapy for solid organ or marrow transplantation.
- Occurs in approximately 1% to 2% of solid-organ transplant recipients.
- There is a clear association between PTLD and the type of organ transplanted.
  — Cardiac-lung and intestinal transplantation have the highest incidence of PTLD.
- The highest incidence occurs in the first years of transplantation.
- The incidence of PTLD after allo-HSCT ranges from 0.5% to 1%.
- The onset of post-transplant lymphoma in most patients is related to B-cell proliferation induced by infection with EBV in the setting of chronic immunosuppression.
- Involvement of the grafted organ occurs in approximately 30% of patients and may lead to organ damage and fatal complications.
- Management of PTLD is not uniform.
  — Reduction of immunosuppression is the first step in the treatment of these patients.
  — Many cases of polyclonal PTLD may resolve completely with a reduction in immunosuppressive therapy.
  — Patients with late PTLD and more aggressive monoclonal PTLD are less likely to respond.
  — Rituximab has shown promising results when incorporated into treatment regimens.
  — The only baseline factor predicting response is a normal level of serum LDH at day 80 of treatment.
- The following sequence is generally recommended:
  — If possible, the first step is reduction in immunosuppression, followed by four weekly cycles of rituximab, if reduction of the immunosuppression is ineffective.
  — If both steps are ineffective, then six cycles of R-CHOP are recommended.

T-CELL–HISTIOCYTE-RICH LARGE B-CELL LYMPHOMA

- This lymphoma is characterized by effacement of the architecture of the lymph node by a lymphohistiocytic infiltrate with a diffuse or vaguely nodular growth pattern.
- It accounts for less than 5% of all cases of DLBCL and occurs at a younger age on average.
- The median age of onset is in the fourth decade.
- A male predominance is noted.
- This subtype more often presents with advanced stage disease, and often in multiple extranodal sites, and with an elevated serum LDH.
- The lymphoma infiltrates the spleen, liver, and marrow with greater frequency than does DLBCL.
- Marrow involvement occurs in approximately one-third of the cases, a frequency considerably higher than in DLBCL, and patients are more likely to develop “B” symptoms than patients with DLBCL.

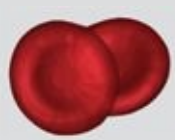
- When treated with CHOP-like regimens, most series suggest that the outcome for these patients is similar to patients with typical DLBCL.
- Six cycles of R-CHOP for advanced disease would be a reasonable initial approach to therapy.

PRIMARY CUTANEOUS DLBCL, LEG TYPE

- Composed solely of large transformed B cells with a predilection for the skin of the leg.
- Primary cutaneous DLBCL, leg type constitutes approximately 4% of all primary cutaneous B-cell lymphomas.
- The median age at the time of presentation is 60 to 70 years.
- Lymphomatous tumors affect the skin of the legs in most cases, but approximately 10% arise at other sites.
- The B cells are usually positive for CD20 and usually express *BCL2* and *FOX-P1*.
- Lymphoma cells often find translocations involving *MYC, BCL6*, or *IGH* genes.
- The gene expression profile of these lymphoma cells is often the same as activated B-cell like DLBCL.
- Anthracycline-containing chemotherapy with rituximab should be considered as initial therapy.

ANAPLASTIC LYMPHOMA KINASE-POSITIVE LARGE B-CELL LYMPHOMA

- This uncommon neoplasm of large immunoblast-like B cells stains for nuclear and or cytoplasmic anaplastic lymphoma kinase (ALK) protein.
- The lymphoma cells may undergo plasmablastic differentiation.
- The average age of presentation is in the fourth decade with a male predilection.
- Most patients present with advanced stage disease.
- The most common affected nodal areas are in the neck and mediastinum.
- Common extranodal involvement includes the liver, spleen, bone, and gastrointestinal tract.
- The lymphoma cells are large immunoblasts with a large central nucleolus.
- The lymphoma cells stain for the ALK protein, usually with a granular cytoplasmic appearance, but nuclear staining may also occur. These cells are usually CD3, CD20, CD30, CD79a negative.
- Occasional cases may have a t(2;17)(p23;q23) that results in a clathrin-ALK fusion protein.
- The clinical course of ALK-positive large B-cell lymphoma is aggressive with a median survival time of 24 months.
- Tumors are frequently negative for CD20, making the utility of rituximab uncertain.



For a more detailed discussion, see Stephen D. Smith and Oliver W. Press: Diffuse Large B-Cell Lymphoma, Chap. 98 in *Williams Hematology*, 9th ed.

CHAPTER 61

Follicular Lymphoma

- Follicular lymphoma (FL) is an indolent lymphoid neoplasm that is derived from mutated germinal center B cells and exhibits a nodular or follicular histologic pattern.
  — FL is typically composed of a mixture of small, cleaved follicle center cells referred to as *centrocytes* and large noncleaved follicular center cells referred to as *centroblasts*.
  — The disease has masqueraded under multiple previous monikers, including "nodular lymphoma" in the Rappaport classification and "follicle center cell lymphoma" in the Working Formulation.
- FL accounts for approximately 20% to 25% of adult non-Hodgkin lymphomas (NHLs) in the United States, with an annual incidence of approximately 14,000 new cases per year.
- The disease is uncommon in persons younger than age 20 years. Pediatric cases appear to represent a separate disease entity that is typically localized, lacks the translocation 14;18 and BCL-2 expression, and has a very good prognosis.

CLINICAL FEATURES

Symptoms and Signs

- Patients with FL usually present with painless diffuse lymphadenopathy.
- Less frequently, patients may have vague abdominal complaints, including pain, early satiety, and increasing girth, which are caused by a large abdominal lymphomatous mass.
- Approximately 10% of patients present with B symptoms (fever, drenching night sweats, or loss of 10% of their body weight).

Staging the Disease

- Evaluation involves performance of a (1) medical history; (2) physical examination (with attention to the lymph nodes in the Waldeyer ring and size and involvement of liver and spleen); (3) laboratory testing, including a complete blood count, examination of the blood film and a differential white cell count, lactic acid dehydrogenase [LDH], β_2-microglobulin, comprehensive metabolic panel, and serum uric acid level; (4) lymph node biopsy; (5) marrow aspiration and biopsy; (6) flow cytometric analysis of blood, marrow, and lymph node cells; and (7) computed tomography (CT) of the chest, abdomen, and pelvis or positron emission tomography (PET)-CT (favored imaging modality).
- Excisional lymph node biopsies are strongly preferred for the initial histologic diagnosis, although in cases in which nodal masses are inaccessible, generous needle core biopsies may suffice.
- The diagnosis should not be established solely on the basis of flow cytometry of the blood or

marrow, or on cytologic examination of aspiration needle biopsies of lymph node or other tissue.

- In selected circumstances, additional CT scans of the neck, PET-CT imaging, measurement of the cardiac ejection fraction, serum protein electrophoresis, quantitative immunoglobulins, and hepatitis C testing may be useful.
- Hepatitis B serology should be done before administering rituximab.

LABORATORY FEATURES

Lymph Node Morphology

- A predominantly nodular lymph node pattern is evident; however, the neoplastic follicles are distorted and as the disease progresses, the malignant follicles efface the nodal architecture (**Figure 61–1**).
- The World Health Organization has developed a three-grade classification system according to the proportion of centroblasts (ie, large noncleaved follicular center cells) detected microscopically:
  — Grade 1: 0 to 5 centroblasts per high-power field
  — Grade 2: 6 to 15 centroblasts per high-power field
  — Grade 3: > 15 centroblasts per high-power field; A, with mixture of centrocytes and centroblasts, and 3B, with sheets of centroblasts.
- Most authorities agree that grade 3B FL behaves aggressively and should be treated with anthracycline-containing regimens (eg, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone [R-CHOP]) similar to diffuse large B-cell lymphoma.

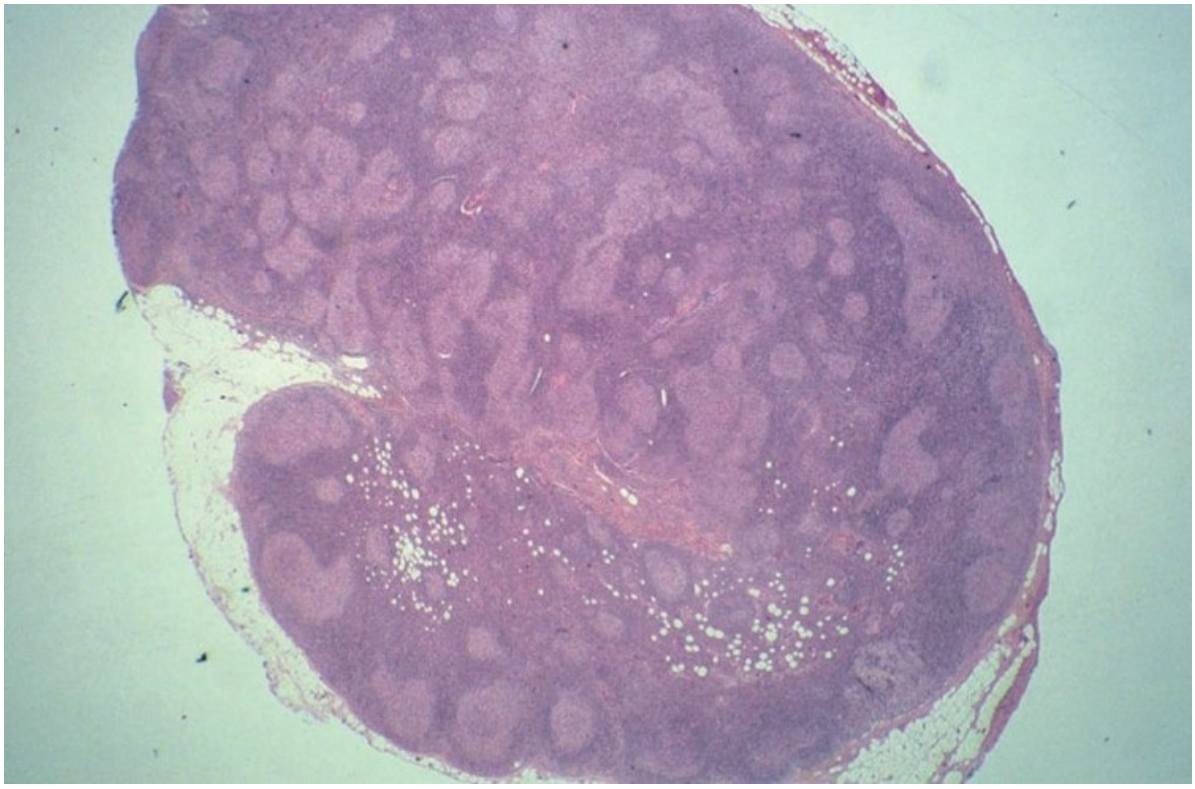


FIGURE 61–1 Lymph node biopsy. Grade 2 follicular lymphoma (low-power magnification). Characteristic replacement of entire node (cortex and medulla) by lymphoid follicles. (Source: *Williams Hematology*, 9th ed, Chap. 96, Fig. 96–17.)

Cytogenetics

- The classic cytogenetic finding is the t(14;18)(q32;q21) translocation that juxtaposes the *BCL-2* gene on band q21 of chromosome 18 with the immunoglobulin (Ig) heavy-chain gene on band 32 of chromosome 14 **(Figure 61–2)**.
  — This translocation occurs in 85% to 90% of cases and in virtually all cases with a grade 1 histopathology (≥ 95% centrocytes).
- The Ig enhancer element results in amplified expression of the translocated gene product and thus overexpression of BCL-2 protein, leading to inhibition of apoptosis of affected B cells **(Figure 61–3)**.
- However, detection of the t(14;18) translocation in lymphoid cells is neither necessary nor sufficient for the diagnosis of FL.
- Additional cytogenetics abnormalities are found in 90% of patients: most commonly, loss of 6q and 17p, and gains of 2, 5, 6p, 7, 8, 12, 17q, 18, 21, and X.

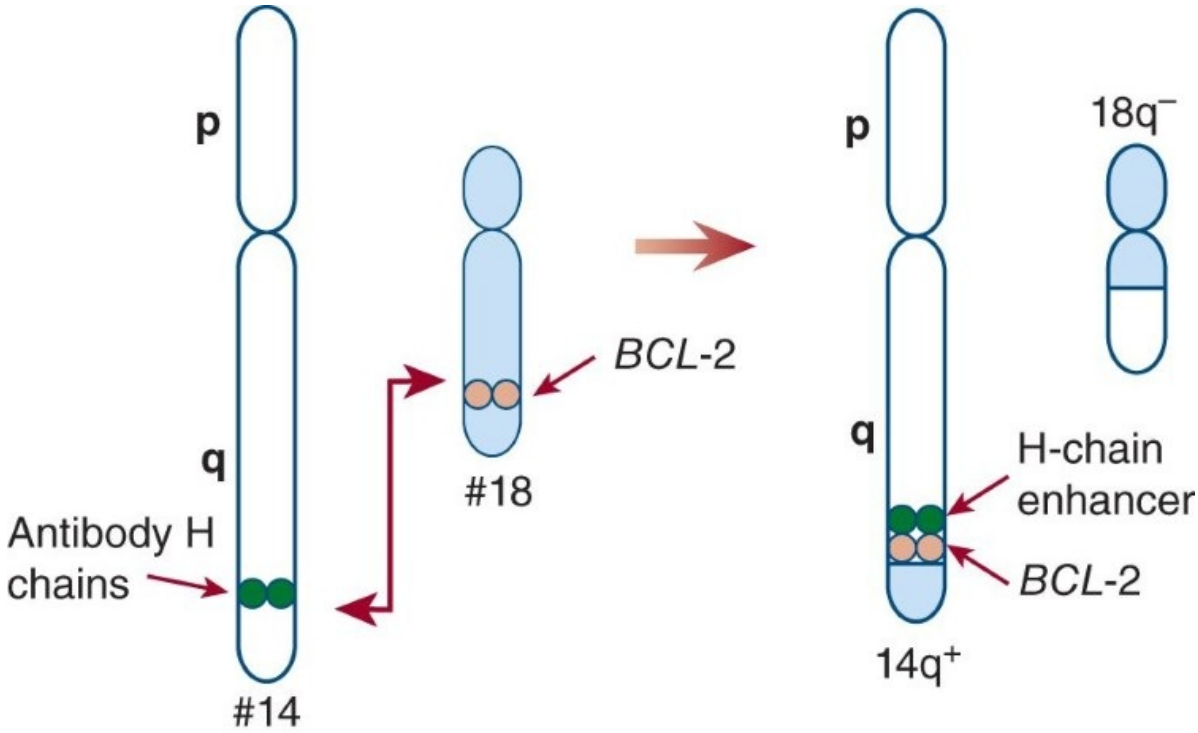
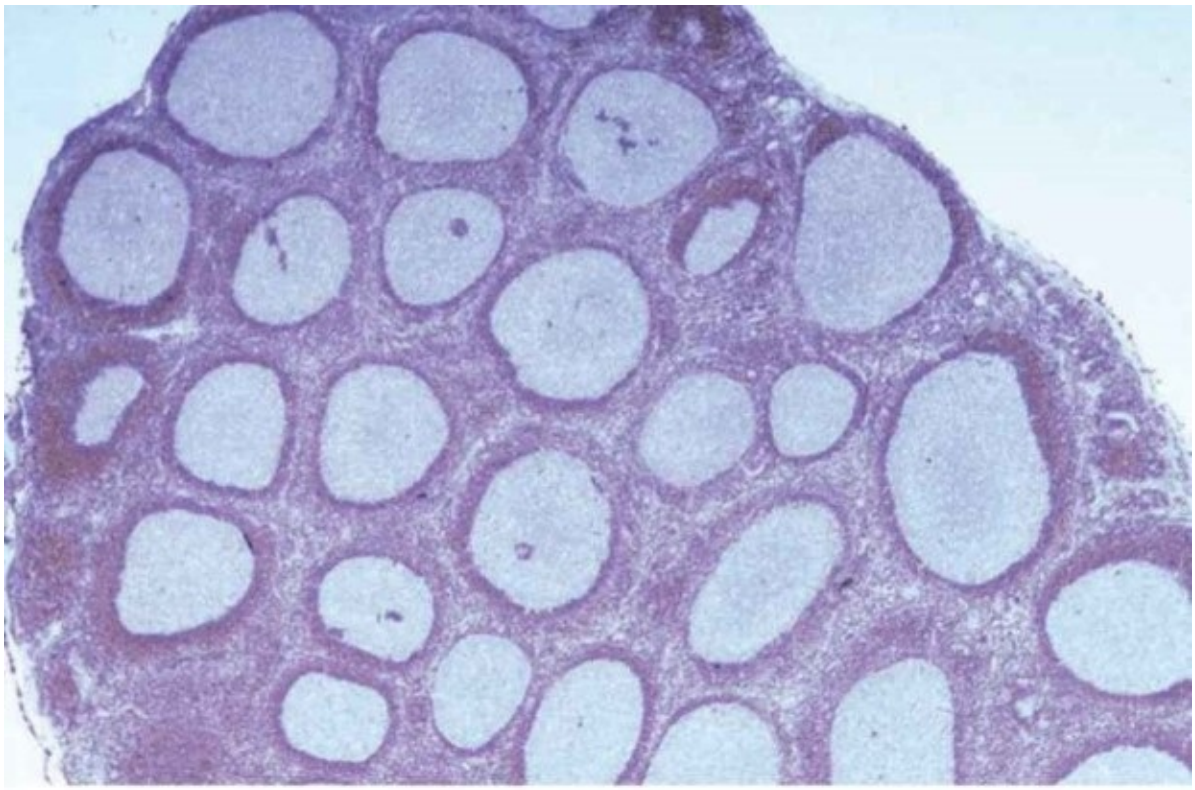
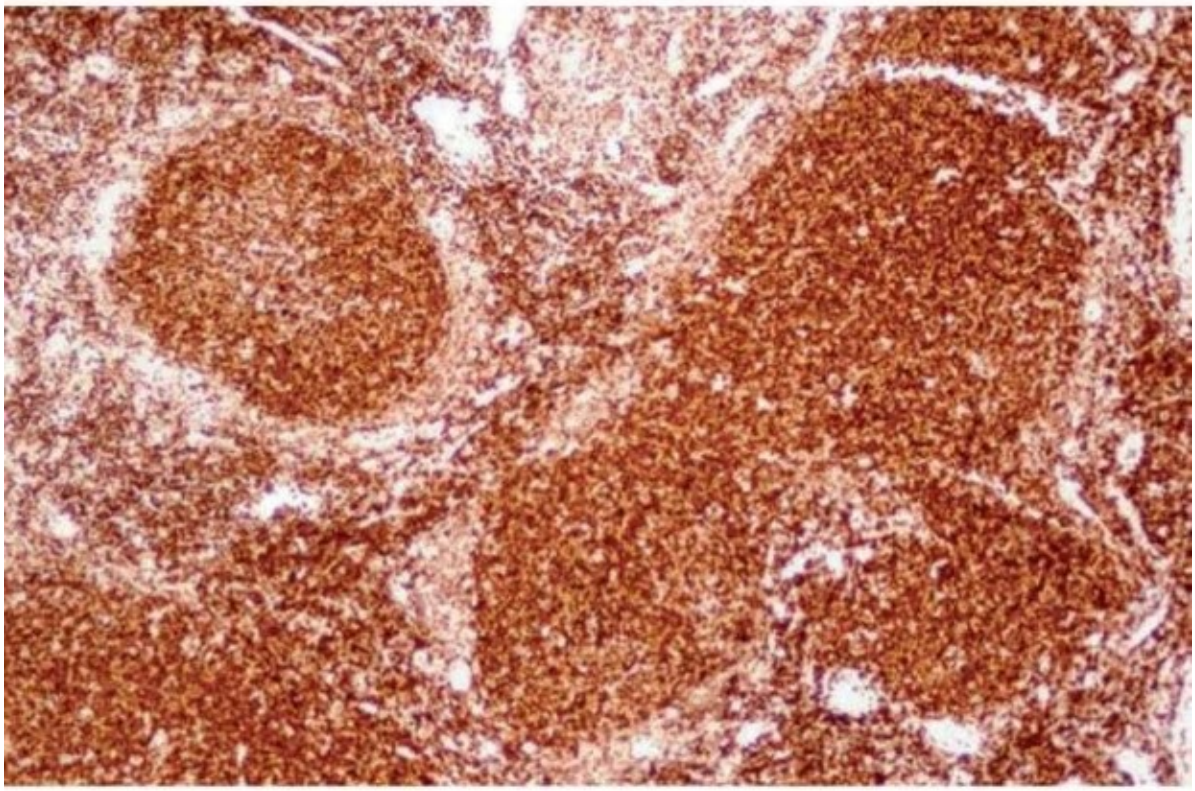


FIGURE 61–2 The t(14;18)(q32;q21) translocation juxtaposes the *BCL*-2 gene on band q21 of chromosome 18 with the immunoglobulin heavy-chain gene on band 32 of chromosome 14. (Source: *Williams Hematology*, 9th ed, Chap. 99, Fig. 99–2.)



A



B

FIGURE 61–3 **A.** Lymph node from patient with reactive follicular hyperplasia stained with an antibody to the antiapoptotic protein BCL2. Note the absence of staining of the germinal centers of the follicles where most of the cells will die during the maturation process. **B.** Lymph node from patient with follicular lymphoma stained with a BCL2 immunostain. Note intense brown stain in the germinal centers indicating presence of large amounts of BCL-2 (Contrast with **A**.) (Source: *Williams Hematology*, 9th ed, Chap. 96, Fig. 96–5A, Fig. 96–20B.)

PROGNOSTIC FACTORS

Clinical and Laboratory Values

- There are five adverse prognostic factors: age (> 60 years vs ≤ 60 years), Ann Arbor stage (III–IV vs I–II), hemoglobin level (< 120 g/L vs ≥ 120 g/L), number of nodal areas (> 4 vs ≤ 4), and serum LDH level (high vs normal).
- Three risk groups are defined: low risk (0–1 adverse factors, 36% of patients), intermediate risk (2 factors, 37% of patients, hazard ratio [HR] of 2.3), and poor risk (≥3 adverse factors, 27% of patients, HR = 4.3). **Figure 61–4** shows outcomes following chemotherapy with and without rituximab.

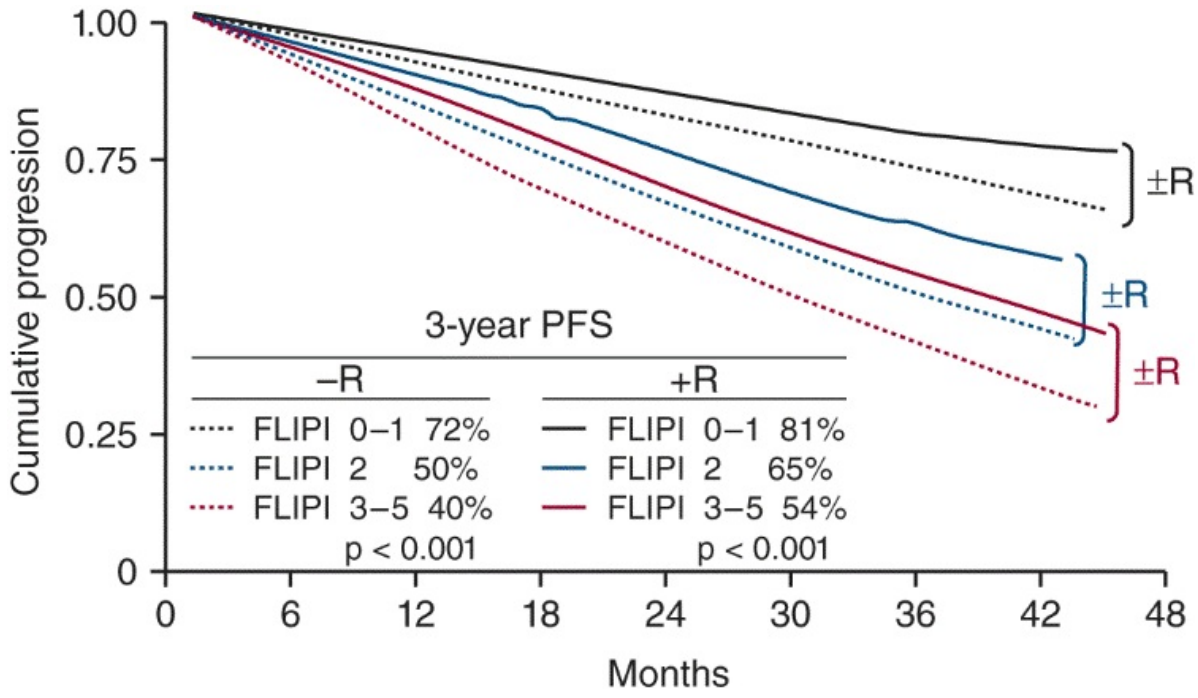


FIGURE 61–4 Progression-free survival (PFS) of 827 patients with FL stratified by the Follicular Lymphoma International Prognostic Index (FLIPI) into low risk (0 to 1 risk factors, 40% of patients, *black lines*), intermediate risk (2 risk factors, 33% of patients, *blue lines*), or high risk (3 to 5 risk factors, 27% of patients, *red lines*). Of the 827 patients, 267 were treated with chemotherapy regimens without rituximab *(dotted lines)* and 560 were treated with rituximab-containing regimens *(solid lines)*. (Data from Federico M, Bellei M, Pro B: Revalidation of FLIPI in patients with follicular lymphoma registered in the F2 study and treated upfront with immunochemotherapy. *Proc Am Soc Clin Oncol* 25:443s, 2007.

Gene Expression Profiling

- Two gene expression signatures allow construction of a survival predictor that enables segregation of patients into four quartiles with disparate median lengths of survival (13.6, 11.1, 10.8, and 3.9 years), independent of clinical prognostic variables.
- One signature (immune response 1) is associated with a good prognosis and includes genes encoding T-cell markers (eg, *CD7*, *CD8B1*, *ITK*, *LEF1*, and *STAT4*) as well as genes that are highly expressed in macrophages (eg, *ACTN1* and *TNFSF13B*).
- The immune response 2 signature is associated with a poor prognosis and includes genes preferentially expressed in macrophages, dendritic cells, or both (eg, *TLR5*, *FCGR1A*, *SEPT10*, *LGMN*, and *C3AR1*).
- Next-generation sequencing has identified mutations in specific epigenetic regulators (eg, MLL2, CREBBP, EP300, and EZH2) in almost all cases.

TREATMENT

Limited Stage I or II

Radiotherapy

- Patients with stage I or II FL represent only 10% to 30% of all cases in most series.
- Standard management for stage I or limited contiguous stage II disease involves the administration of involved field radiotherapy (35–40 Gy).
- Adjuvant chemotherapy does not appear to improve survival in this setting.

Observation (Watch and Wait)

- Excellent survival has also been observed in patients with early-stage disease who received no initial therapy.
- In a group of 43 selected patients, 56% were free from the requirement for treatment for at least 10 years and 86% were alive 10 years after diagnosis.
- Based on this study, many authorities have concluded that "watchful waiting" is an acceptable alternative to radiotherapy for stage I or II FL.

Advanced Stage II to IV

Observation (Watch and Wait)

- Because there is no conclusive evidence that survival of FL patients is improved by immediate institution of therapy, or that conventional management (other than allogeneic hematopoietic stem cell transplantation) can cure the disease, a "watch-and wait" approach is also recommended for patients with asymptomatic extensive stage II or stage III or IV FL.

Single-Agent Chemotherapy

- Patients can be palliated effectively with a variety of single chemotherapy agents (**Table 61–1**).

TABLE 61–1 THERAPEUTIC REGIMENS FOR FOLLICULAR LYMPHOMA

Agent(s)	Dose	Route	Days(s) of Treatment	Repeat Cycle at Day
SINGLE AGENTS				
Chlorambucil	0.08–0.12 mg/kg	PO	Daily	
	or 0.4–1.0 mg/kg	PO	1	28
Cyclophosphamide	50–100 mg/m^2	PO	Daily	
	or 300 mg/m^2	PO	1–5	28
Fludarabine	25 mg/m^2/d	IV	1–5	28
Cladribine	0.1 mg/kg/d	IV (continuous)	1–7	28
	or 0.14 mg/kg/d	IV (2 h)	1–5	28
Bendamustine	70–120 mg/m^2/d	IV	1, 2	21 or 28

Rituximab	375 mg/m^2/d	IV	1, 8, 15, 22	
COMBINATION THERAPY				
Stanford CVP				
Cyclophosphamide	400 mg/m^2	PO	1–5	21
Vincristine	1.4 mg/m^2 (maximum 2 mg)	IV	1	21
Prednisone	100 mg/m^2	PO	1–5	21
R-CVP				
Rituximab	375 mg/m^2	IV	1	21
Cyclophosphamide	750–1000 mg/m^2	IV	1	21
Vincristine	1.4 mg/m^2 (maximum 2 mg)	IV	1	21
Prednisone	100 mg	PO	1–5	21
R-CHOP				
Rituximab	375 mg/m^2	IV	1	21
Cyclophosphamide	750 mg/m^2	IV	1	21
Doxorubicin	50 mg/m^2	IV	1	
Vincristine	1.4 mg/m^2	IV	1	
Prednisone	100 mg	PO	1–5	
FND				
Fludarabine	25 mg/m^2	IV	1–3	28
Mitoxantrone	10 mg/m^2	IV	1	
Dexamethasone	20 mg	IV or PO	1–5	

Source: *Williams Hematology*, 9th ed, Chap. 99, Table 99–1.

Monoclonal Antibody Therapy

- Four weekly infusions of rituximab were administered at a dose of 375 mg/m^2 to patients with FL. The response rate was 48%, including a 6% complete response rate and a median time to progression of approximately 1 year.
- Rituximab as initial and maintenance therapy has an overall response rate (ORR) of approximately 70%, with a complete response rate of approximately 20% and a median progression-free survival of 34 months. Ongoing or maintenance rituximab prolongs remission duration.
- New monoclonal anti-CD20 antibodies being studied include ofatumumab, veltuzumab, and obinutuzumab.
- A radioimmunoconjugates ^{90}yttrium (^{90}Y)-ibritumomab tiuxetan (Zevalin) is approved by the US Food and Drug Administration for relapsed, refractory, and transformed indolent lymphomas.
- In a randomized study comparing treatment of patients with relapsed FL with either ^{90}Y-ibritumomab tiuxetan or rituximab, the ORR (8% vs 55%) and the complete response (CR) rate (30% vs 15%) were both statistically superior in the group treated with the

radioimmunoconjugate.

- The major concern with the use of ^{90}Y-ibritumomab tiuxetan therapy is the potential for delayed myelosuppression. Growth factor administration and transfusions are required in approximately 20% of patients. A potential long-term concern with radiolabeled antibody formulations is the potential development of myelodysplasia and acute leukemia as late complications.

Combination Chemotherapy and Rituximab

- In one study, induction therapy consisting of eight cycles of rituximab/cyclophosphamide, vincristine, and prednisone (R-CVP) was compared with eight cycles of CVP without rituximab in 321 patients with newly diagnosed disease. R-CVP was superior to CVP alone in terms of ORR (81% vs 57%), CR rate (41% vs 10%), time to progression (32 months vs 15 months), time to treatment failure (27 months vs 7 months), and overall survival (OS: 83% vs 77% at 4 years, $P = .029$).
- Similarly, R-CHOP was compared with CHOP for first-line treatment of 428 patients with advanced stage FL. R-CHOP exhibited a superior ORR (96% vs 90%), time to treatment failure ($P < .001$), duration of response ($P = .001$), and OS ($P = .016$) compared with CHOP alone.
- Randomized trials have shown that R-CHOP and R-fludarabine containing regimens both have a superior progression-free survival (PFS) than R-CVP. Because fludarabine-containing regimens have more hematological toxicity and a greater risk of secondary malignancies, R-CHOP has generally been thought to be the preferred regimen.
- Bendamustine-rituximab has been shown to have similar efficacy and less toxicity than R-CHOP, and it is frequently utilized.

Maintenance Rituximab

- The PRIMA study showed that 2 years of maintenance rituximab yielded a higher 2-year PFS (75% vs 58%) than no ongoing rituximab. However, there was no improvement in OS in this or other trials.

Idiotype Vaccines

- Phase 2 trials showed favorable results with vaccines directed against the idiotypic immunoglobulin protein expressed on the lymphoma cells.
- However, phase 3 trials have failed to confirm the phase 2 results.

Hematopoietic Stem Cell Transplantation

- The role of high-dose chemoradiotherapy and allogeneic hematopoietic stem cell transplantation in the management of patients remains controversial. This lack of clear advantage may be related to its application usually in refractory or relapsed advanced stage patients.
- When allogeneic and autologous hematopoietic stem cell transplant results are compared in patients with relapsed FL, the long-term survival rates are comparable.

- Adverse outcomes of autologous hematopoietic stem cell transplantation include treatment-related mortality (3%–5%) and a substantial increase in the incidence of secondary myelodysplasia and acute myelogenous leukemia, occurring in approximately 15% of patients.
- Although allogeneic hematopoietic transplantation affords long-term PFS for approximately 40% to 50% of patients with relapsed disease, transplant-related mortality rates range from 20% to 40%. Nonmyeloablative and reduced-intensity allogeneic transplant conditioning regimens to exploit the benefit of a graft-versus-lymphoma effect while minimizing transplant morbidity and mortality show promising results.
- Adverse outcomes associated with autologous hematopoietic stem cell transplantation include treatment-related mortality (3%–5%) and a substantial increase in the incidence of secondary myelodysplasia and acute myelogenous leukemia, occurring in 7% to 19% of patients.
- Although allogeneic hematopoietic transplantation affords long-term PFS for approximately 40% to 50% of patients with relapsed disease, transplant-related mortality rates range from 20% to 40% and the usual advanced age of patients.

Transformed Follicular Lymphoma

- As many as 30% to 40% of patients with FL transform to diffuse large B-cell lymphoma (Table 61–2) with a poor survival after transformation (Table 61–3).
- Transformation often presents as explosive growth of nodes in one nodal or extranodal site.
- Autologous transplantation may yield better outcomes than immunochemotherapy.

TABLE 61–2 TRANSFORMATION OF FOLLICULAR LYMPHOMA TO A MORE RAPIDLY PROGRESSIVE HISTOPATHOLOGICAL TYPE (CUMULATIVE FRACTION OF 325 PATIENTS FOLLOWED FROM DIAGNOSIS)

Years	Cumulative Fraction
3	0.18
6	0.21
9	0.28
12	0.34
15	0.40
18	0.40
24	0.40
34	0.40

Data derived from Montoto S, Davies AJ, Matthews J, et al. Risk and clinical implications of transformation of follicular lymphoma to diffuse large B-cell lymphoma. *J Clin Oncol* 25(17):2426–2433, 2007. Cumulative fraction extrapolated from Source: *Williams Hematology*, 9th ed, Chap. 99, Figure 99-6.

TABLE 61–3 PROPORTION OF PATIENTS WITH FOLLICULAR LYMPHOMA SURVIVING AFTER TRANSFORMATION TO A LESS-FAVORABLE HISTOPATHOLOGICAL PATTERN

Year From Transformation	Fractional Survival after Transformation (Fraction of 88 Patients Followed)
0	1.00
3	.31
6	.27

9	.21
12	.13
15	.06
20	.06
26	.06

Data from Montoto S, Davies AJ, Matthews J, et al.: Risk and clinical implications of transformation of follicular lymphoma to diffuse large B-cell lymphoma. *J Clin Oncol* 25(17):2426–2433, 2007. Fractional survival extrapolated from source: *Williams Hematology*, 9th ed., Chap. 99, Figure 99-6.

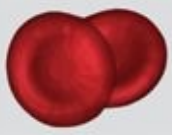
COURSE AND PROGNOSIS

- The OS of patients with FL is improving (**Table 61–4**).

TABLE 61–4 IMPROVED SURVIVAL OF PATIENTS WITH FOLLICULAR LYMPHOMA

Cohort	Years after Registration	Percent Survival
1970s	3	78
	6	59
	9	43
1980s	3	87
	6	69
	9	58
1995-present	3	95
	6	89
	9	--

Data from Fisher RI, LeBlanc M, Press OW, et al: New treatment options have changed the survival of patients with follicular lymphoma. *J Clin Oncol* 23(33):8447-8452, 2005. Percent survival extrapolated from source: *Williams Hematology*, 9th ed., Chap. 99, Figure 99-7.



For a more detailed discussion, see Oliver W. Press: Follicular Lymphoma, Chap. 99 in *Williams Hematology*, 9th ed.

CHAPTER 62

Mantle Cell Lymphoma

- Mantle cell lymphoma (MCL) cells display an immunophenotype similar to lymphocytes in the mantle zone of normal germinal follicles, surface immunoglobulin (sIg) M+, sIgD+, CD5+, CD20+, CD10–, CD43+. In contrast to chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma (SLL), MCL cells typically do not express CD23.
- MCL had been previously classified as an intermediate-grade lymphoma and called *intermediate lymphocytic lymphoma* and *centrocytic lymphoma.* MCL can be confused with other types of lymphoma or leukemia, such as SLL, CLL, or marginal zone lymphoma.

PATHOPHYSIOLOGY

- Insight into the pathophysiology of MCL was realized with discovery of the cytogenetic abnormality t(11;14) (q13;q32) in the tumor cells, a translocation resulting in the overexpression of the cell cycle regulator cyclin D1.
- However, it is almost certain that additional genetic events are involved in development of the fully transformed state because low numbers of cells carrying the t(11;14) translocation have been found in the blood of some healthy individuals without any evidence of disease.
- The *ATM* (ataxia-telangiectasia mutant) gene is mutated in approximately 40% of patients. *ATM* inactivation facilitates genomic instability in lymphoma cells through an impaired response to DNA damage.
- Additional genetic anomalies that could contribute to the disease include losses in chromosomes 1p13-p31, 2q13, 6q23-27, 8p21, 9p21, 10p14-15, 11q22-23, 13q11-13, 13q14-34, 17p13, and 22q12; gains in chromosomes 3q25, 4p12-13, 7p21-22, 8q21, 9q22, 10p11-12, 12q13, and 18q11q23; and high copy-number amplifications of certain chromosomal regions.

CLINICAL FEATURES

- The typical presentation is that of an older patient with lymphadenopathy in several sites (eg, cervical, axillary, inguinal). There is a male predominance.
- Patients may be asymptomatic, but a significant proportion may have fever, night sweats, or weight loss.
- The liver may be enlarged, and the spleen is enlarged in 40% of patients at the time of diagnosis. Gastrointestinal involvement is common with multiple colonic polyps (ie, polyposis coli), a characteristic presentation seen in a minority of patients. As many as 90% of patients will have a positive result of a blind colon biopsy.
- A number of adverse prognostic features of MCL have been identified, including the

expression of the Ki67 proliferation antigen in a high proportion of lymphoma cells, high serum level of β_2-microglobulin in the absence of renal dysfunction, high serum levels of lactic acid dehydrogenase (LDH), presence of blastoid cytology, advanced patient age, Ann Arbor advanced stage, extranodal presentation, and constitutional symptoms, among others.

- A prognostic model called the Mantle Cell International Prognostic Index (MIPI) has been introduced, which uses four independent prognostic factors: age, performance status, LDH, and leukocyte count.
- There is no consensus on the risk of central nervous system (CNS) disease in patients with MCL or the need to give CNS prophylaxis. Studies have reported an incidence of CNS of 4% and a 5-year actuarial risk of 26%.
- The major presenting characteristics of patients with mantle cell lymphoma are shown in **Table 62–1**.

TABLE 62–1 PATIENT CHARACTERISTICS AT PRESENTATION*

Characteristic	Number
Age (years)	
< 60	123
> 60	178
Sex	
Male	230
Female	71
Stage	
I–II	23
III–IV	267
Status (World Health Organization)	
0–1	233
≥ 2	43
Lactate dehydrogenase	
Elevated	56
Normal	140
International Prognostic Index	
0–1	15
≥ 2	75
Marrow involvement	
Yes	207
No	81
B symptoms	
Yes	107
No	155
Extranodal involvement	
Yes	161

No	16

*304 cases.

Data from Tiemann M, Schrader C, Klapper W, et al: European MCL Network: Histopathology, cell proliferation indices and clinical outcome in 304 patients with mantle cell lymphoma (MCL): A clinicopathological study from the European MCL Network. *Br J Haematol* 131(1):29–38, 2005.

LABORATORY FEATURES

- Approximately 50% of patients present with blood and marrow involvement, sometimes with an overt leukemic phase, but more often with subtle involvement with detection of the malignant lymphocyte immunophenotype by flow cytometry of blood or marrow cells.
- Almost all cases of MCL show overexpression of cyclin D1 mRNA. The rare cases that are negative for cyclin D1 usually overexpress cyclin D2 or D3.
- MCL cells stain strongly for the antiapoptotic molecule BCL-2 and are negative for the germinal center markers CD10 and BCL-6.

DIAGNOSIS

- The diagnosis is made by biopsy interpreted by a hematopathologist.
- The immunophenotype of MCL has some similarities to that of CLL or SLL.
- In contrast to CLL or SLL, MCL cells react strongly with FMC7, a weak anti-CD20 monoclonal antibody, and typically do not express CD23.
- The t(11;14) is characteristic.
- All cases of MCL express cyclin D1, typically at levels that are much higher than that of other lymphomas (**Figure 62–1**).

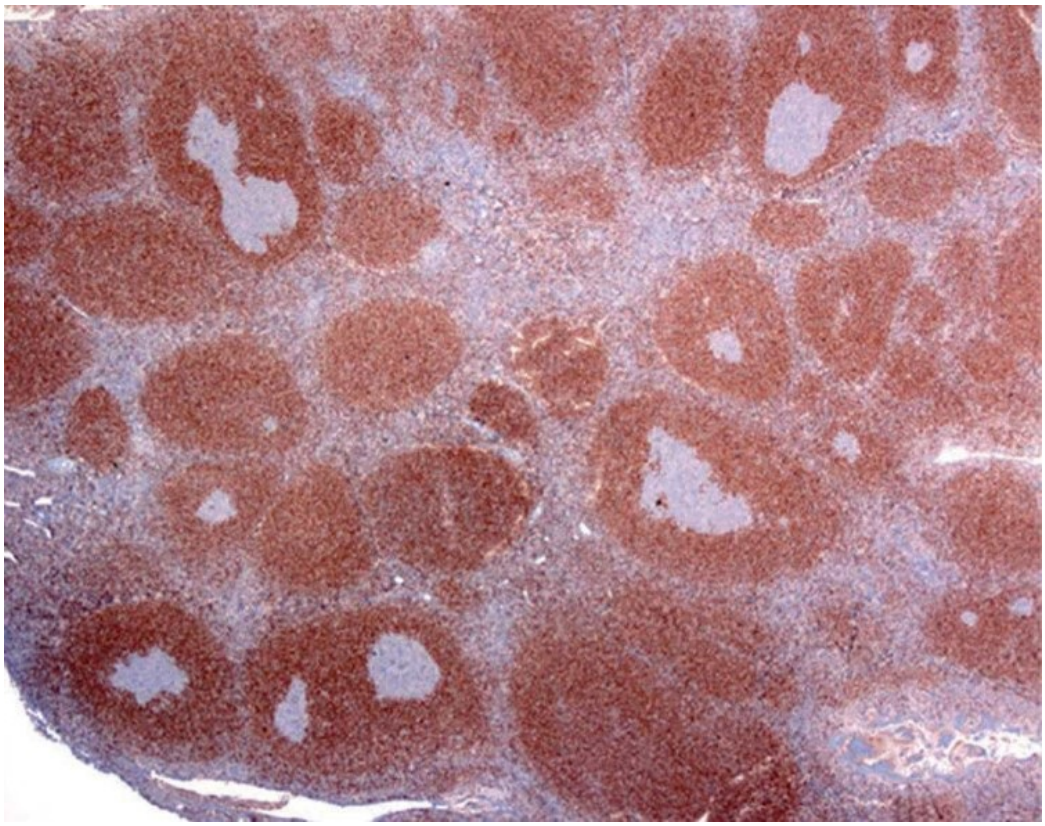


FIGURE 62–1 Biopsy of node in patient with mantle cell lymphoma stained for cyclin D1. Note rust colored positive reaction product staining expanded follicle mantle zones. Some pale gray, unstained, germinal centers are evident in some follicles. (Source: *Williams Hematology*, 9th ed, Chap 96, Fig. 96–16.)

PRIMARY TREATMENT

- Localized disease is rare. Because of the presence of advanced disease at presentation, most patients require systemic therapy.
- The anti-CD20 monoclonal antibody rituximab is the most important recent addition to the therapy of lymphomas, including MCL. Rituximab alone produces an approximate 40% response in patients with untreated MCL. However, the lymphoma cells of some patients can lose expression of CD20 after repeated therapy.
- In patients with blood involvement, the first dose of rituximab should be infused slowly and with close monitoring during initial therapy because of the risk of tumor lysis syndrome or cytokine-release syndrome.
- A few patients have an indolent presentation and do not require therapy for up to several years; these patients often present with marrow involvement and splenomegaly with lymphadenopathy.
- For the rare patient with localized disease a shortened course of chemotherapy and radiotherapy may be appropriate.
- Immunochemotherapy is the standard of care. Characteristic regimens are shown in **Table 62–2**.
- The type of regimen used should be determined in part by the patient's age, performance status, and possible comorbid conditions.
- Use of intensified therapeutic regimens in patients who would be predicted to tolerate such approaches has also been studied as described in **Table 62–3**. Autologous stem cell

transplantation has been incorporated into some of these treatment programs.

TABLE 62–2 CONVENTIONAL IMMUNOCHEMOTHERAPY FOR MANTLE CELL LYMPHOMA

Author (Year)	Phase	Number of Patients	Regimen	ORR% (CR%)	Median PFS (Months)	2-Year OS (%)
Howard (2002)	II	40	R-CHOP	96 (48)	17	95 (3 years)
Lenz (2005)	III	112	CHOP R-CHOP	75 (7) 94 (34)	14 (TTF) 21 (TTF)	77 77
Herold (2008)	III	90	MCP R-MCP	63 (15) 71 (32)	18 20	52 (4 years) 55 (4 years)
Gressin (2010)	II	113	R-VAD-C	73 (48)	18 (no ASCT) 58 (ASCT)	62 (3 years)
Sachenes (2011)	II	20	R-chlorambucil	95 (90)	89% (3 years)	95 (3 years)
Kenkra (2011)	II	22	R-hyperCVAD R maintenance	77 (64)	38	62 (4 years)
Kluin-Nelemans (2012)	III	485	R-CHOP R-FC	86 (34) 78 (40)	28 (TTF) 26 (TTF)	62 (4 years) 47 (4 years)
		274	I maintenance	NA	29% (4 year DR)	67 (4 years)
			R maintenance		58% (4 year DR)	79 (4 years)
Smith (2012)	II	50	R-CHOP ^{90}Y-ibritumomab	64 (46)	31 (TTF)	73 (5 years)
Rummel (2013)	III	94	R-CHOP BR	91 (30) 93 (40)	21 35	No difference
Visco (2013)	II	20	R-BAC	100 (95)	95% (2 years)	93
Ruan (2011)	II	35	R-CHOP + bortezomib	91 (72)	44% (2 years)	86
Houot (2012)	II	29	R-doxorubicin/ dexamethasone/ chlorambucil + bortezomib	79 (59)	26	69
Chang (2014)	II	75	R-hyperCVAD + bortezomib R maintenance	95 (68)	67% (3 years)	91 (3 years)
Robak (2015)	III	487	R-CHOP			
			R-CHP + bortezomib	89 (53)	14	54 (4 years)
				92 (42)	25	64 (4 years)
Inwards (2014)	I	17	R-cladribine + temsirolimus	94 (53)	19	65

ASCT, autologous stem cell transplantation; BR, bendamustine and rituximab; CR, complete response; MCP, mitoxantrone, chlorambucil, and prednisolone; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; R-BAC, rituximab, bendamustine, and cytarabine; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; R-CHP, rituximab, cyclophosphamide, doxorubicin, and prednisone; R-FC, rituximab, fludarabine, and cyclophosphamide; R-hyperCVAD, rituximab, hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone; R-MCP, rituximab, mitoxantrone, chlorambucil, and prednisone; TTF, time to treatment failure; ^{90}Y, yttrium-90.
Source: *Williams Hematology*, 9th ed, Chap. 100, Table 100–2.

TABLE 62–3 DOSE-INTENSIFIED IMMUNOCHEMOTHERAPY OF MANTLE CELL LYMPHOMA

	Number of	Median PFS

Author (Year)	Phase	Patients	Regimen	ORR% (CR%)	(Years)	OS (%)
Dreyling (2005)	III	122 R-CHOP + ASCT R-CHOP + IFN		98 (81) 99 (37)	3.3 1.4	83 (3 years) 77 (3 years)
De Guibert (2006)	II	17 R-DHAP + ASCT 7 R-DHAP		100 (94) 86 (86)	76% (3 years) NA	75% (3 years) NA
Damon (2009)	II	77	R-CHOP/MTX/Ara-C/etoposide + ASCT	88 (69)	56% (5 years)	64 (5 years)
van 't Veer (2009)	II	87	R-CHOP/Ara-C + ASCT	70 (64)	36% (4 years)	56 (4 years)
Magni (2009)	II	28	Sequential R-chemo + ASCT	100 (100)	57% (low risk) 34% (high risk)	76 (low risk) 68 (high risk)
Geisler (2012)	II	160	R-maxiCHOP/Ara-C + ASCT	96 (54)	7.4	58 (10 years)
Hermine (2012)	III	455 R-CHOP + ASCT R-CHOP/DHAP + ASCT		97 (61) 98 (63)	3.8 7.3	67 (5 years) 74 (5 years)
Delarue (2013)	II	60	R-CHOP/DHAP + ASCT	100 (96)	6.9	75 (5 years)
Le Gouill (2014)	III	299 R-DHAP + ASCT R-DHAP + ASCT + R maintenance		NA NA	83% (2 years) 93% (2 years)	93% (2 years) 95% (2 years)
Romaguera (2010)	II	97	R-hyperCVAD	97 (87)	4.5	64 (10 years)
Merli (2012)	II	60	R-hyperCVAD	83 (72)	61% (5 years)	73 (5 years)
Bernstein (2013)	II	2013	R-hyperCVAD	86 (55)	4.8	63 (5 years)

Ara-C, cytarabine; ASCT, autologous stem cell transplantation; CR, complete response; DHAP, dexamethasone, high-dose cytarabine, and cisplatinum; IFN, interferon; MTX, methotrexate; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; R, rituximab; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; R-DHAP, rituximab, dexamethasone, high-dose cytarabine, and cisplatinum; R-hyperCVAD, rituximab, hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone; R-maxiCHOP, cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisone.
Source: *Williams Hematology*, 9th ed, Chap. 100, Table 100–3.

Salvage Therapy

- For patients with relapsed/refractory disease, a wide variety of effective regimens are available, depending on the previous therapy the patient has received (**Table 62–4**).
- Several new agents have shown activity in patients with relapsed MCL including bortezomib, lenalidomide, temsirolimus, everolimus, ibrutinib, idelalisib, and Venetoclax (**Table 62–5**).

TABLE 62–4 CONVENTIONAL IMMUNOCHEMOTHERAPY OF RELAPSED MANTLE CELL LYMPHOMA

Author (Year)	Phase	Number of Patients	Regimen	ORR% (CR%)	Median PFS (Months)	Median OS (Months)
Forstpointner (2004)	III	24 24	FCM R-FCM	46 (0) 58 (29)	4 8	11 65% (2 years)
Rummel (2005)	II	16	BR	75 (50)	18	NA
Robinson (2008)	II	12	BR	92 (42)	19	NA

Weide (2007)	II	18	BMR	78 (33)	21	60% (2 years)
Gironella (2012)	II	28	R-GemOx	79 (75)	18	30
Visco (2013)	II	20	R-BAC	80 (70)	87% (2 years)	93% (2 years)
Weigert (2009)	Retrospective	8	R-Ara-C + bortezomib	50 (25)	5	16
Ruan (2010)	II	22	R-PEP-C + bortezomib	73 (32)	10	45% (2 years)
Kouroukis (2011)	II	25	Gemcitabine + bortezomib	60 (11)	11	NA
Friedberg (2011)	II	7	BR + bortezomib	71 (NA)	NA	NA
Gerecitano (2011)	II	10	R-CP + bortezomib	60 (50)	NA	NA
Furtado (2015)	II	46	CHOP CHOP + bortezomib	48 (22) 83 (35)	8 17	12 36
Ruan (2010)	II	22	R-PEP-C + thalidomide	73 (32)	10	45% (2 years)
Zaja	II	42	BR + lenalidomide	79 (55)	68% (1 year)	82% (1 year)
Hess (2015)	I	11	BR + temsirolimus	91 (45)	22	92% (19 months)

BR, bendamustine and rituximab; BMR, bendamustine, mitoxantrone, rituximab; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; CR, complete response; FCM, fludarabine, cyclophosphamide, and mitoxantrone; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; R-Ara-C, rituximab and cytarabine; R-BAC, rituximab, bendamustine, and cytarabine; R-CP, rituximab, cyclophosphamide, and prednisone; R-FCM, rituximab, fludarabine, cyclophosphamide, and mitoxantrone; R-GemOx, rituximab, gemcitabine, and oxaliplatin; R-PEP-C, rituximab, prednisone, etoposide, procarbazine, and cyclophosphamide.
Source: *Williams Hematology*, 9th ed, Chap. 100, Table 100–4.

TABLE 62–5 THERAPY FOR RELAPSED MANTLE CELL LYMPHOMA

Author (Year)	Phase	Number of Patients	Regimen	ORR% (CR%)	Median PFS (Months)	Median OS (Months)
Goy (2009)	II	141	Bortezomib	33 (8)	7	24 months
Baiocchi (2011)	II	13	R-bortezomib	29 (29)	2	NA
Lamm (2011)	II	16	R-bortezomib/ dexamethasone	81 (44)	12	39
Kaufmann (2004)	II	16	R-thalidomide	81 (31)	20	75% (3 years)
Zinzani (2013)	II	57	Lenalidomide	35 (12)	4	19
Goy (2013)	II	134	Lenalidomide	28 (88)	4	19
Trneny (2014)	II	170 84	Lenalidomide monochemotherapy	40 (5) 11 (0)	9 5	28 21
Wang (2012)	II	44	R-lenalidomide	57 (36)	11	24
Witzig (2005)	II	34	Temsirolimus 250 mg	38 (3)	7 (TTP)	12
Ansell (2008)	II	27	Temsirolimus 25 mg	41 (4)	6 (TTP)	14
Hess (2009)	III	162	Temsirolimus 175/75 mg Temsirolimus 175/25 mg chemotherapy	22 (2) 6 (0) 2 (2)	5 3 2	13 10 10
Ansell (2011)	II	69	R-temsirolimus	59 (19)	10	30
Renner (2012)	II	35	Everolimus	20 (6)	6	NA
Wang (2013)	II	111	Ibrutinib	68 (21)	14	NA
Wang (2014)	II		R-ibrutinib	100 (low Ki-67)	90% (1 year)	90% (1 year)

				50 (high Ki-67)	13.6	13.6
Kahl (2014)	I	16	Idelalisib	62 (NA)	3 (RD)	NA
Davids (2013)	I	8 MCL	Abt-199 (Venetoclax)	100	NA	NA

CR, complete response; NA, not available; ORR, overall response rate; OS, overall survival; PFS progression-free survival; R, rituximab; TTP, time to progression.
Source: *Williams Hematology*, 9th ed, Chap. 100, Table 100–5.

PROGNOSIS

- MCL is currently considered incurable, but long-term remissions can occur (**Figure 62–2**).

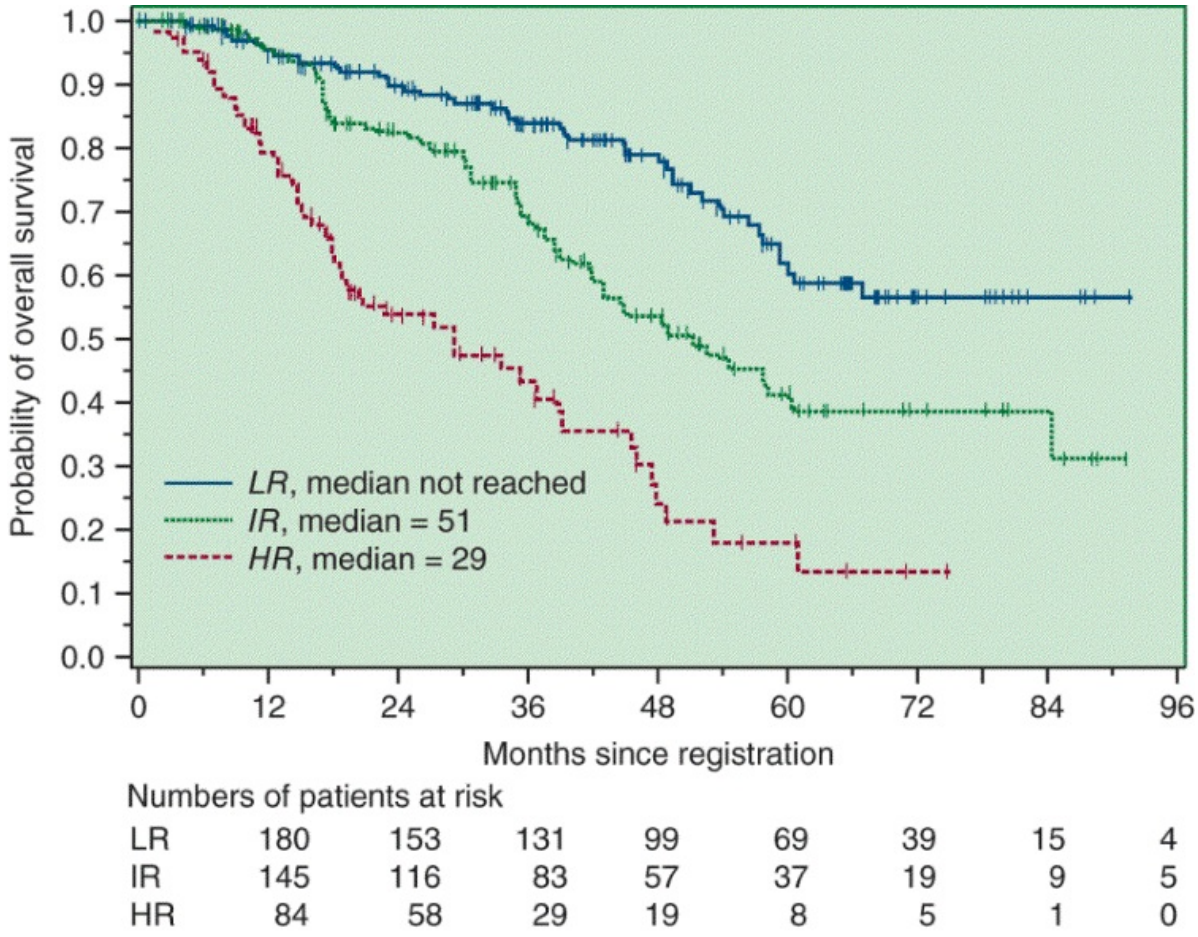
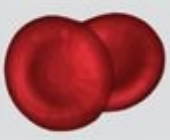


FIGURE 62–2 Overall survival according to the Mantle Cell International Prognostic Index (MIPI). *LR* indicates low risk, prognostic score less than 5.7; *IR* indicates intermediate risk, prognostic score 5.7 or more but less than 6.2; and *HR* indicates high risk, prognostic score 6.2 or more. The prognostic score is calculated as [0.03535 × age (years)] + 0.6978 (if ECOG > 1) + [1.367 × $\log_{10}$(LDH/ULN)] + [0.9393 × $\log_{10}$(WBC count)]. ECOG, Eastern Cooperative Oncology Group performance status score; LDH, lactic acid dehydrogenase; ULN, upper limits of normal; WBC, white blood cell. (Reproduced with permission from Hoster E, Dreyling M, Klapper W, et al: A new prognostic index (MIPI) for patients with advanced-stage mantle cell lymphoma. German Low Grade Lymphoma Study Group (GLSG): European Mantle Cell Lymphoma Network, *Blood* 2008;Jan 15;111(2):558-565.)



For a more detailed discussion, see Martin Dreyling: Mantle Cell Lymphoma, Chap. 100, in *Williams Hematology*, 9th ed.

CHAPTER 63
Marginal Zone B-Cell Lymphoma

- The marginal zone lymphomas (MZLs) are derived from memory-type or antigen-experienced B cells that reside in regions contiguous to the outer part of the mantle zones of B-cell follicles.
- The World Health Organization (WHO) defines three separate MZL entities, namely, the extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (currently known as MALT lymphoma), the nodal marginal zone B-cell lymphoma (previously termed *monocytoid lymphoma*), and the splenic marginal zone B-cell lymphoma (with or without circulating villous lymphocytes).
- Gastric MALT lymphoma is one of the best examples of a microbiologic (*Helicobacter pylori*) cause of a human malignancy.

PATHOPHYSIOLOGY

- MALT lymphomas arise from mucosa-associated lymphoid tissues in the context of chronic inflammation.
- In addition to *H pylori*, other bacteria are possibly implicated in the pathogenesis of MZLs arising in the skin (*Borrelia burgdorferi*), in the ocular adnexa (*Chlamydophila psittaci*), and in the small intestine (*Campylobacter jejuni*).
- Hepatitis C virus (HCV) appears involved in the pathogenesis of splenic MZL through antigen-driven stimulation of the lymphoma clone.
- There is, however, a great geographic variation in the strength of these associations, which is not satisfactorily explained.
- An increased risk of developing MALT lymphoma has been also reported in individuals affected by autoimmune disorders, especially Sjögren syndrome and systemic lupus erythematosus.
- Several recurrent chromosomal translocations have been described in extranodal MZLs. Three of them—[t(11;18)(q21;q21), t(1;14)(p22;q32), and t(14;18)(q32;q21)]—are the best characterized; they each affect the same signaling pathway, activating nuclear factor-kappa B (NF-κB), a transcription factor that plays a major role in immunity, inflammation, and apoptosis (**Table 63–1**).

TABLE 63–1 COMMON GENETIC LESIONS IN MUCOSA-ASSOCIATED LYMPHOID TISSUE LYMPHOMA

	Lesion	Genes	Frequency	Sites
Translocations	t(11;18)(q21;q21)	*BIRC3-MALT1*	15%–40%	Stomach, lung
	t(14;18)(q32;q21)	*IGHV-MALT1*	20%	Lung, skin, ocular adnexa, salivary

				gland
	t(1;14)(p22;q32)	*IGHV-BCL10*	< 5%	Stomach, lung
	t(3;14)(p13;q32)	*IGHV-FOXP1*	< 5%	Unclear
Gains	+3; +3q		20%–40%	No differences in sites
	+18; +18q		20%–40%	No differences in sites
Losses	–6q23	*TNFAIP3*	15%–30%	No differences in sites

BCL-10, B-cell CLL/lymphoma 10 gene; *BIRC3*, baculoviral IAP repeat-containing 3 gene; *FOXP1*, forkhead box P1 gene; *IGHV*, immunoglobulin heavy-chain variable region gene; *MALT1*, mucosa-associated lymphoid tissue translocation gene 1; *TNFAIP3*, tumor necrosis factor-α–induced protein 3 gene.
Source: *Williams Hematology*, 9th ed, Chap. 101, Table 101–1.

EXTRANODAL MARGINAL ZONE LYMPHOMAS

Clinical Features

- The most common site of MALT lymphoma is the stomach, representing at least one-third of all cases.
- Extranodal MZLs may also arise at many other sites, including the salivary gland, the thyroid, the upper airways, the lung, the ocular adnexa (lacrimal gland, conjunctiva, eyelid, orbital soft tissue), the breast, the liver, the urogenital system, the skin and other soft tissues, and even the dura.
- As a general rule, the presenting symptoms of extranodal MZLs are related to the primary location (eg, dyspepsia, pain, and nausea for gastric MALT lymphoma and nodules/papules for cutaneous MALT lymphomas).
- Elevated serum lactic acid dehydrogenase (LDH) or serum β_2-microglobulin levels, as well as constitutional B symptoms, are rare at presentation.
- MALT lymphoma can remain localized for a prolonged period within the tissue of origin, but regional lymph nodes can sometimes be infiltrated and dissemination at multiple sites occurs in up to one-fourth of cases. Transformation to a high grade lymphoma is unusual.
- Marrow or lymph node involvement is seen in 25% of cases or less.
- Immunoproliferative small intestinal disease (a special variant of intestinal MALT lymphoma) usually presents with severe, unremitting signs and symptoms of malabsorption.

Diagnosis

- The presence *H pylori* is determined by histochemistry or, alternatively, the urea breath test.
- The B cells of MZLs show the immunophenotype of the normal marginal zone B cells present in spleen, Peyer patches, and in lymph nodes. Therefore, the tumor B cells express surface immunoglobulins and pan-B antigens (CD19, CD20, and CD79a), express the marginal zone-associated antigens CD35 and CD21, and lack expression of CD5, CD10, CD23, or high-level expression of cyclin D1.
- The tumor cells of extranodal MZL typically express immunoglobulin (Ig) M, less often IgA, or IgG, whereas splenic zone lymphoma is typically IgD-positive.
- In addition to standard histology and immunohistochemistry, fluorescence in situ hybridization analysis or use of polymerase chain reaction for detection of t(11;18) may be useful for

identifying patients who are unlikely to respond to antibiotic therapy for *H pylori*.

Staging

- The initial staging procedures for a gastric MALT lymphoma should include a gastroduodenal endoscopy with multiple biopsies taken from each region of the stomach, duodenum, gastroesophageal junction, and from any abnormal-appearing site.
- Endoscopic ultrasound is recommended to evaluate the regional lymph nodes and gastric wall infiltration.
- Other recommended laboratory and radiologic studies include measurement of serum LDH and β_2-microglobulin; computed tomography of the chest, abdomen, and pelvis; and marrow aspirate and biopsy.
- Multiorgan involvement is not uncommon and complete staging procedures are recommended for patients with nongastric mantle zone B-cell lymphoma.

Treatment

- Eradication of *H pylori* with antibiotics plus proton-pump inhibitor regimens should be the sole initial treatment of localized (ie, confined to the stomach) *H pylori*–positive gastric MALT lymphoma. *H pylori* eradication results in complete regression of gastric MALT lymphoma in approximately two-thirds of cases.
- Treatment of *H pylori* is based on triple or quadruple therapy, including a proton-pump inhibitor, clarithromycin and amoxicillin or metronidazole for 14 days, or a proton-pump inhibitor, metronidazole, tetracycline, and bismuth subcitrate for 14 days.
- Histologic evaluation of repeat biopsies remains an essential follow-up procedure, with multiple biopsies taken 2 to 3 months after treatment to document that the lymphoma is not progressing and that *H pylori* eradication has been achieved.
- Patients who do not respond or have only a partial response to antibiotic therapy should be considered for radiotherapy.
- Because gastric MALT lymphoma is multifocal, the surgical procedure is a total gastrectomy with its associated complications. Surgery has not been shown to achieve superior results in comparison with organ-preserving strategies.
- Alternatively, excellent disease control can be achieved with involved field radiotherapy alone for stages I and II MALT lymphoma of the stomach without evidence of *H pylori* infection or with persistent lymphoma after antibiotic eradication.
- The optimal management of nongastric disease is not clearly established and should be "patient-tailored," taking into account the site, the stage, and the clinical characteristics of the individual patient.
- In general, the treatment used for *H pylori*–negative cases can be applied to nongastric MALT lymphoma. Radiation therapy is considered the treatment of choice for localized lesions. MALT lymphomas at different sites have been successfully eradicated with involved field radiation therapy encompassing the involved organ alone with doses of approximately 30 to 36 Gy.
- Patients with systemic disease should be considered for systemic chemotherapy and/or immunotherapy with anti-CD20 monoclonal antibodies (**Table 63–2**).

TABLE 63–2 **CHEMOTHERAPY/IMMUNOTHERAPY EXPERIENCES IN GASTRIC MUCOSA-ASSOCIATED LYMPHOID TISSUE LYMPHOMA**

Study	Patients	Early Stage	Treatment	Outcomes
Hammel	24	71%	Cyclophosphamide or chlorambucil	75% CR
Avilés	83	100%	CHOP × 3 + CVP × 4	100% CR
Jäger	19	100%	Cladribine	100% CR
Martinelli	27	86%	Rituximab	46% CR; 31% PR
Raderer	7	57%	R-CHOP/R-CNOP	100% CR
Conconi	13	100%	Bortezomib	46% CR; 15% PR
Salar	21	64%	Bendamustine + rituximab	94% CR; 6% PR

CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; CNOP, cyclophosphamide, mitoxantrone, vincristine, prednisone; CR, complete response; CVP, cyclophosphamide, vincristine, prednisolone; PR, partial response; R, rituximab.
Source: *Williams Hematology*, 9th ed, Chap. 101, Table 101–2.

SPLENIC MARGINAL ZONE LYMPHOMA

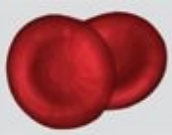
- This mature B-cell neoplasm involves white pulp follicles in the spleen, splenic hilar nodes, marrow, and blood.
- This very rare splenic MZL comprises less than 1% of all lymphomas. More than half of cases present with circulating villous lymphocytes with characteristic fine, short cytoplasm polar projections. When these are more than 20% of the lymphocyte count, the term *splenic lymphoma with villous lymphocytes* is commonly used.
- This condition is often associated with hepatitis C infection, and antiviral treatment can sometimes induce remission.
- Most common genetic abnormalities are 3q trisomy and gains of 12q.
- Patients usually present with isolated splenomegaly, cytopenias, and lymphocytosis without palpable lymphadenopathy.
- Autoimmune anemia or thrombocytopenia is seen in 10% to 15% of patients, and a monoclonal protein is frequent.
- It can be difficult to distinguish from lymphoplasmacytic lymphoma.
- Asymptomatic patients can be observed.
- Splenectomy is an excellent initial therapy and can result in prolonged remissions.
- Single-agent rituximab or rituximab combined with chemotherapy is also effective and the preferred initial therapy by some authorities.
- Transformation to diffuse large B-cell lymphoma is seen in 10% to 20% of cases.

NODAL MARGINAL ZONE LYMPHOMA

- This condition is rare and less well defined than the other MZLs, representing less than 2% of all NHLs.
- The most common genetic abnormality is gain of several regions of chromosome 3.
- Patients usually present with peripheral and abdominal lymphadenopathy.

— Marrow involvement is seen in less than half of patients, and blood involvement is unusual.
— Ten percent of patients have a serum monoclonal immunoglobulin.

- Differentiation from follicular lymphoma and lymphoplasmacytic lymphoma can be difficult.
- No specific treatment guidelines exist. Patients are usually treated in a manner similar to follicular lymphoma.



For a more detailed discussion, see Pier Luigi Zinzani and Alessandro Broccoli: Marginal Zone B-cell Lymphomas, Chap. 101 in *Williams Hematology*, 9th ed.

CHAPTER 64

Burkitt Lymphoma

DEFINITION AND EPIDEMIOLOGY

- Burkitt lymphoma (BL) may present in three distinct forms: endemic (African), sporadic, and immunodeficiency-associated.
- The endemic form is found in eastern equatorial Africa, with a peak age incidence at 4 to 7 years, and it is nearly twice as frequent in boys as in girls.
- Sporadic BL, defined as cases outside of endemic African regions, accounts for 1% to 2% of all patients with non-Hodgkin lymphoma (NHL).
- The incidence is higher in males than in females, and the median age of onset is 30 years.
- Immunosuppression-related BL increased in incidence during the AIDS epidemic.

PATHOPHYSIOLOGY

- The unifying feature of all three types of BL is activation of the c-*MYC* gene, typically resulting from translocations involving the long arm of chromosome 8, which carries c-*MYC*.
  - — Such translocations commonly involve also the long arm of chromosome 14, which carries the immunoglobulin heavy chain gene complex, but might instead involve chromosome 2 or 22, which carries the immunoglobulin kappa or lambda light chain gene complex, respectively.
  - — The constitutive activation of c-*MYC* increases the expression of a number of genes encoding proteins involved in cell proliferation.
- Translocations are thought to occur via double-strand breaks that occur during the normal B-cell class-switch reaction and somatic hypermutation.
- Up to one-third of cases also might have alterations involving the short arm of chromosome 17 at 17p13.1, involving the *TP53* gene encoding p53. Loss of p53 function might be selected in BL cells that otherwise would be induced to undergo apoptosis in response to overexpression of c-*MYC*.
- Next-generation sequencing has found frequently occurring mutations in *ID3*, *TCF3*, and *CCND3*.
- Evidence of Epstein-Barr virus (EBV) is found in essentially all patients with African BL, one-third of patients with immunosuppression associated BL, and in one-fifth of patients with nonendemic form of disease.

CLINICAL FEATURES

- The endemic (African) form often presents as a jaw or facial bone tumor. It may spread to extranodal sites, especially to the marrow and meninges. Almost all cases are EBV positive.
- The nonendemic or American form presents as an abdominal mass in approximately 65% of cases, often with ascites. Extranodal sites, such as the kidneys, gonads, breast, marrow, and central nervous system (CNS) may be involved. Involvement of the marrow and CNS is much more common in the nonendemic form.
- Tumor lysis syndrome is very common following induction chemotherapy but also can occur spontaneously prior to therapy, especially in patients with a high tumor burden. Spontaneous tumor lysis is a poor prognostic indicator.
- The syndrome results in some or all of the following: hyperuricemia and hyperuricosuria, hyperkalemia, hyperphosphatemia, hypocalcemia, metabolic acidosis, and uric acid nephropathy with renal failure as a result of the extraordinary proliferative nature of BL cells.

LABORATORY FEATURES

- Patients with bulky disease may have BL cells in marrow and blood with accompanying suppression of normal blood counts.
  — Marrow involvement is characteristically extensive with replacement by Burkitt-type lymphoblasts with a very high nuclear:cytoplasmic ratio, deep blue rim of cytoplasm, and often prominent cellular vacuolization (**Figure 64–1**).
- Rare cases, often males, may present principally with marrow and blood involvement, so-called Burkitt cell leukemia.
- The serum lactic acid dehydrogenase is often elevated as a reflection of the high cell turnover, especially in patients with bulky disease.
- BL cells are mature B cells that typically express CD19, CD20, CD22, CD79a, and surface IgM. BL cells lack expression of CD5 or CD23.

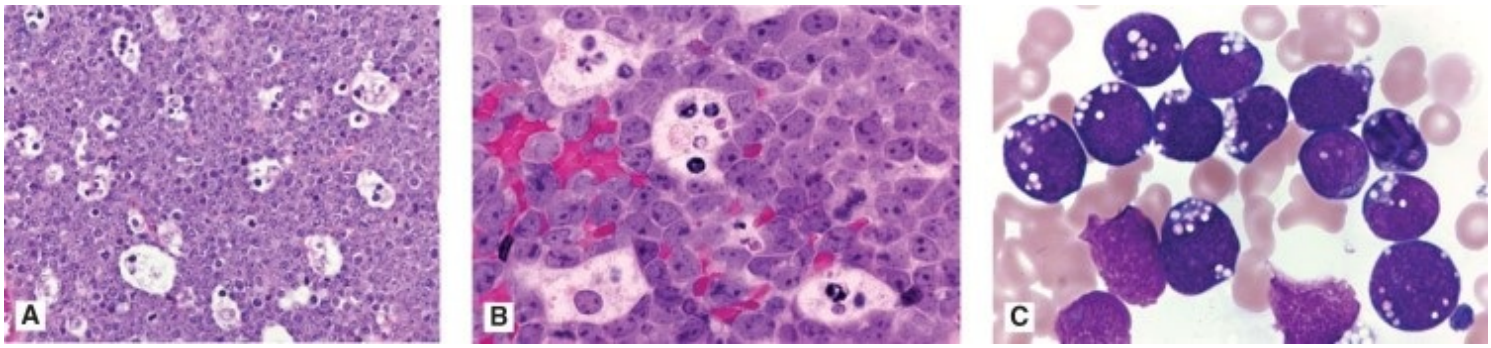


FIGURE 64–1 Burkitt marrow involvement. **A.** Marrow biopsy showing diffuse infiltration of lymphoblasts with interspersed macrophages engorged with cellular debris (tingible-body macrophages). This pattern in marrow or lymph node has been called the "starry sky appearance," a characteristic of the histopathology of Burkitt lymphoma. **B.** Higher magnification of marrow biopsy. **C.** Marrow aspirate showing characteristically vacuolated Burkitt cells. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

DIAGNOSIS

- All cases of BL have a translocation between the long arm of chromosome 8, the site of the c-*MYC* proto-oncogene (8q24), and one of three translocation partners: the Ig heavy-chain

region on chromosome 14, the κ light-chain locus on chromosome 2, or the λ light-chain locus on chromosome 22.

- The translocations involving *c-MYC* can be detected by fluorescence in situ hybridization (FISH) (**Figure 64–2**).

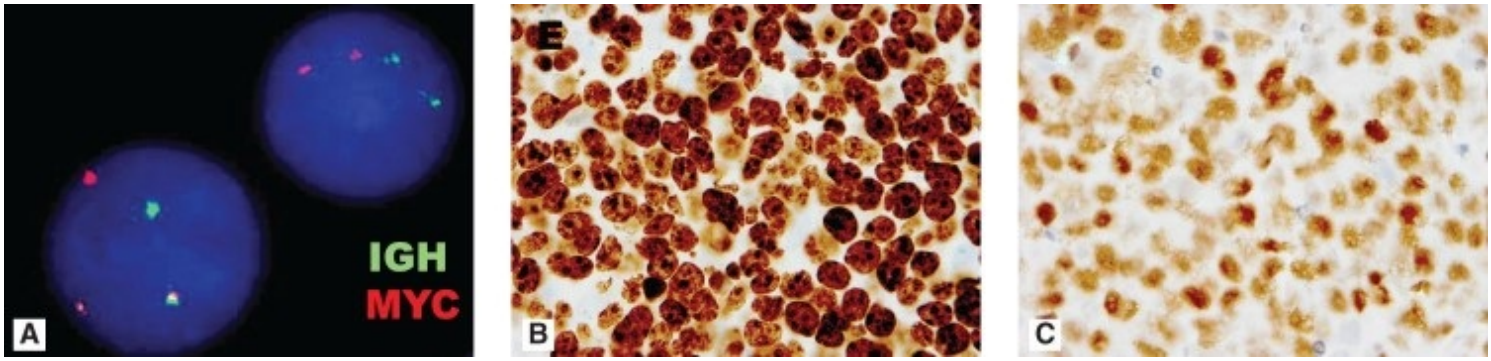


FIGURE 64–2 **A.** Fluorescence in situ hybridization image demonstrating the presence of *IGH-MYC* translocation (lower left hand cell). Colocation of *IGH* probe, labeled green, with MYC probe, labeled red, results in a fused yellow signal representing the *IGH-MYC* oncogene, consistent with the t(8;14) chromosomal translocation. The upper right hand cell shows the normal pattern of two red and two green, nontranslocated alleles. **B.** Ki-67 immunoperoxidase stain of Burkitt cells. Virtually all nuclei stain with the reddish-brown reaction product indicating the high prevalence of cells in the mitotic cell cycle. **C.** The immunoperoxidase stain for the MYC product (brown color) indicating the upregulated nuclear expression in virtually all cells. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

STAGING

- Although virtually all lymphoma is staged with a uniform set of parameters, staging in BL employs a distinct system (**Table 64–1**).

TABLE 64–1 MURPHY STAGING SYSTEM FOR BURKITT LYMPHOMA

Stage I: Single nodal or extranodal site excluding mediastinum or abdomen
Stage II: Single extranodal tumor with regional nodal involvement
Two extranodal tumors on one side of diaphragm
Primary gastrointestinal tumor with or without associated mesenteric nodes
Two or more nodal areas on one side of diaphragm
Stage IIR: Completely resected intra-abdominal disease
Stage III: Two single extranodal tumors on opposite sides of diaphragm
All primary intrathoracic tumors
All paraspinal or epidural tumors
All extensive primary intra-abdominal disease
Two or more nodal areas on opposite sides of diaphragm
Stage IIIA: Localized, nonresectable abdominal disease
Stage IIIB: Widespread multiorgan abdominal disease
Stage IV: Initial central nervous system or marrow involvement (< 25%)

Reproduced with permission from Perkins AS, Friedberg JW: Burkitt lymphoma in adults, *Hematology Am Soc Hematol Educ Program* 2008:341-348.

TREATMENT

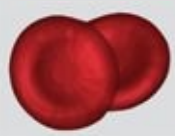
- BL is a highly aggressive tumor; however, therapy with multiagent chemotherapeutic programs results in excellent long-term remission rates and long-term survival of up to 85% of children.

- Risk stratification allows patients with limited disease to be treated with less intensive therapy than more advanced cases and still achieve very high responses.
- Regimens used for BL and treatment results are found in **Table 64–2**.
- There are no studies directly comparing regimens, with most employing cyclophosphamide, doxorubicin, vincristine, methotrexate, ifosfamide, etoposide, high-dose cytarabine, rituximab, but always with intrathecal chemotherapy.
- In general, shorter durations of chemotherapy (ie, 6 months or less) are as good as longer (18 months) periods of treatment.
- BL has a high proliferative rate, so subsequent chemotherapy cycles should be started as soon as hematologic recovery occurs. Waiting for a fixed period between cycles may lead to regrowth of resistant tumor cells between cycles.
- Given the high proliferative rate of the tumor, and the effect of chemotherapy, upfront treatment for tumor lysis syndrome should occur, especially in patients with a high lactic acid dehydrogenase level or bulky disease.
  — Carefully monitored hydration (~3 liters of saline per day)
  — Allopurinol or rasburicase, the latter especially useful in high-risk or spontaneous tumor lysis cases because of rapid onset of action
  — Continuous venovenous hemofiltration, which has been very useful in permitting concomitant full-dose chemotherapy, while preventing lysis syndrome and renal failure
- In the highly active antiretroviral therapy era, HIV-positive patients with BL should be treated similarly to immunocompetent patients.

TABLE 64–2 OUTCOME OF BURKITT LYMPHOMA IN LARGER STUDIES

Citation	Regimen	No. of Patients	2-Year Outcome
Hoelzer	Short duration/dose intensive; pediatric NHL based	35	51% (estimated survival)
Magrath	CODOX-M/IVAC	54	89% (actual survival)
Mead	CODOX-M/IVAC	58	64% (progression-free survival)
Rizzieri	Short duration/dose intensive with rituximab	105	74% (3-year event-free survival)
Thomas	Hyper-CVAD with rituximab	31	89% (estimated survival)
Dunleavy	Dose adjusted R-EPOCH	29	95% (event-free survival)
Evens	R-CODOX-M/IVAC	25	80% (progression-free survival)

CODOX-M/IVAC, cyclophosphamide, doxorubicin, vincristine, methotrexate, ifosfamide, etoposide and high-dose cytarabine, with intrathecal cytarabine and methotrexate; hyper-CVAD, fractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone; NHL, non-Hodgkin lymphoma; R-CODOX-M/IVAC, rituximab with CODOX-M/IVAC; R-EPOCH, etoposide, vincristine and doxorubicin, with bolus rituximab, cyclophosphamide and steroids.
Source: *Williams Hematology*, 9th ed, Chap. 102, Table 102–2.



For a more detailed discussion, see Andrew G. Evans and Jonathan W. Friedberg: Burkitt Lymphoma, Chap. 102 in *Williams Hematology*, 9th ed.

CHAPTER 65
Cutaneous T-Cell Lymphoma

DEFINITION

- Mycosis fungoides and its variant Sézary syndrome, the two principal types of cutaneous T-cell lymphoma (CTCL), are malignant proliferations of mature memory T lymphocytes of the phenotype CD4+CD45RO+ (memory T cells), which invariably involve the skin.
- Other types of lymphoma may also have prominent skin involvement (see **Table 65–1**).
- In cases of CTCL in which erythrodermia is a prominent feature, the major considerations are shown in **Table 65–2**.

TABLE 65–1 WORLD HEALTH ORGANIZATION–EUROPEAN ORGANIZATION FOR RESEARCH AND TREATMENT OF CANCER CLASSIFICATION OF PRIMARY CUTANEOUS T-CELL AND NATURAL KILLER CELL LYMPHOMAS

I. Mycosis Fungoides (MF) A. MF variants and subtypes 1. Folliculotropic MF 2. Pagetoid reticulosis 3. Granulomatous slack skin
II. Sézary Syndrome
III. Adult T-Cell Leukemia/Lymphoma
IV. Primary Cutaneous CD30+ Lymphoproliferative Disorders A. Primary cutaneous anaplastic large cell lymphoma B. Lymphomatoid papulosis
V. Subcutaneous Panniculitis-Like T-Cell Lymphoma
VI. Extranodal Natural Killer/T-Cell Lymphoma, Nasal Type
VII. Primary Cutaneous Peripheral T-Cell Lymphoma, Unspecified A. Primary cutaneous aggressive epidermotropic CD8+ T-cell lymphoma (provisional) B. Cutaneous γδ T-cell lymphoma (provisional) C. Primary cutaneous CD4+ small-/medium-size pleomorphic T-cell lymphoma (provisional)
VIII. Precursor Hematologic Neoplasm A. CD4+/CD56+hematodermic neoplasm (blastic NK-cell lymphoma)

Source: *Williams Hematology*, 9th ed, Chap. 103, Table 103–1.

TABLE 65–2 CLASSIFICATION OF ERYTHRODERMIC CUTANEOUS T-CELL LYMPHOMA

Erythrodermic Subset (T4)	Preexisting MF	Blood
Sézary syndrome	Rarely	Leukemia: B2
Erythrodermic mycosis Fungoides	Always	Normal or minimally abnormal: B0–B1
Erythrodermic cutaneous T-cell lymphoma, not otherwise	Absent	Normal or minimally abnormal: B0–B1

specified

MF, mycosis fungoides.

Source: *Williams Hematology*, 9th ed, Chap. 103, Table 103–4.

EPIDEMIOLOGY

- CTCL is more common in males than females.
- Median age at diagnosis is 55 years.
- In the United States, there are approximately 3000 cases per year, representing about 3% of lymphomas.
- Americans of African descent have a higher incidence and more progressive disease than Americans of European descent.
- Asians and Hispanics are much less often affected.
- The etiology is unknown.

CLINICAL FINDINGS

- Patients usually present with nonspecific skin lesions (chronic dermatitis) occurring years before diagnosis.
- Early in disease, patients are often diagnosed with eczema (spongiotic dermatitis), psoriatic-like dermatitis, or other nonspecific dermatoses associated with pruritus.
- Histologic diagnosis may be difficult in early stages. Neoplastic infiltrates may be minimal, masked by normal inflammatory cells, and the neoplastic mature CD4+ phenotype may be misinterpreted as normal inflammatory cells.
- Mycosis fungoides may be divided into patch stage (patch-only disease), plaque stage (both patches and plaques) and tumor stage (more than one tumor along with patches and plaques).
- A *patch* is defined as a flat lesion with varying degrees of erythema with fine scaling; a *plaque* is defined as a demarcated, erythematous, brownish lesion, with variable scaling of at least 1 mm elevation above the skin surface; and a *tumor* extends at least 5 mm above the surface (tumors are usually in a setting of patches and plaques) (**Figure 65–1**).
- Lesions have a predisposition for skin folds and non–sun-exposed areas (bathing-trunk distribution), but in later stages they can be generalized and involve the face, palms, soles, and other areas.
- Progression through stages usually occurs over years, but some cases may present with advanced stage lesions.
- Pruritus may be mild or severe and is one of the principal quality-of-life issues for patients. It can lead to insomnia, depression, and suicidal ideation.
- Erythrodermic skin involvement occurs in about 5% of patients and can be slight to severe. It can be associated with scaling, keratoderma, painful fissures in the hands and feet, and nail dystrophy and loss. Severely inflamed skin can lead to bacterial infection, fever, chills, and septicemia.
- Sézary syndrome describes patients with pruritus, generalized exfoliative erythroderma, lymphadenopathy, and CD4+ lymphocytes with hyperconvoluted nuclei in the blood (**Figures**

65–2 and 65–3). It has the worst prognosis of the various types of CTCL.

- Depending on the stage of presentation, lymphadenopathy and other organ involvement may occur.
- Lymphadenopathy is usually evident in 50% of patients at diagnosis and increases as disease progresses.

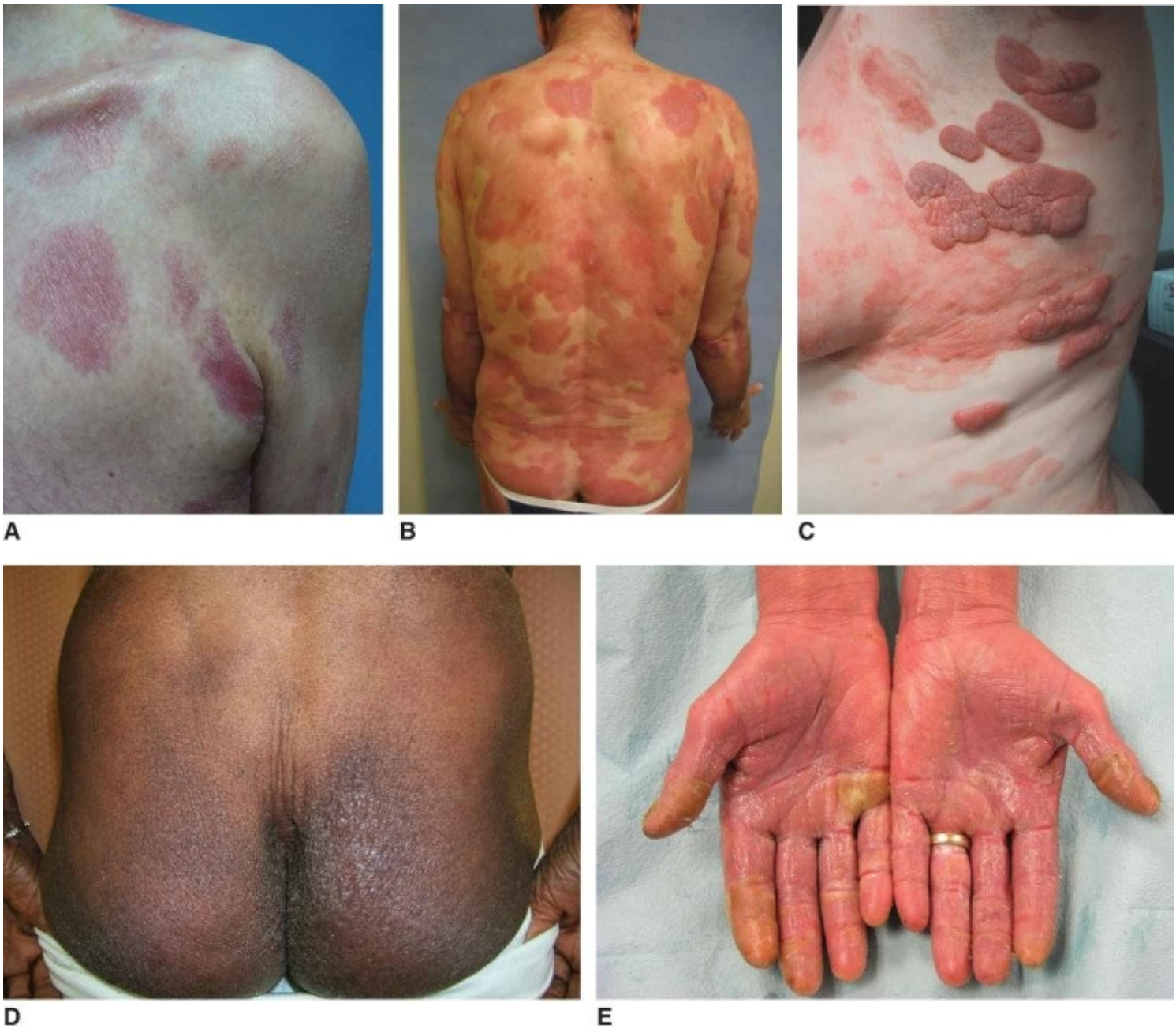


FIGURE 65–1 Mycosis fungoides. **A.** Erythematous atrophic patches with fine scale. **B.** Extensive patches and thicker plaques. **C.** Tumors on the background of preexisting patches and plaques. **D.** Poikiloderma. **E.** Keratoderma. (Source: *Williams Hematology*, 9th ed, Chap. 103, Fig. 103–1.)

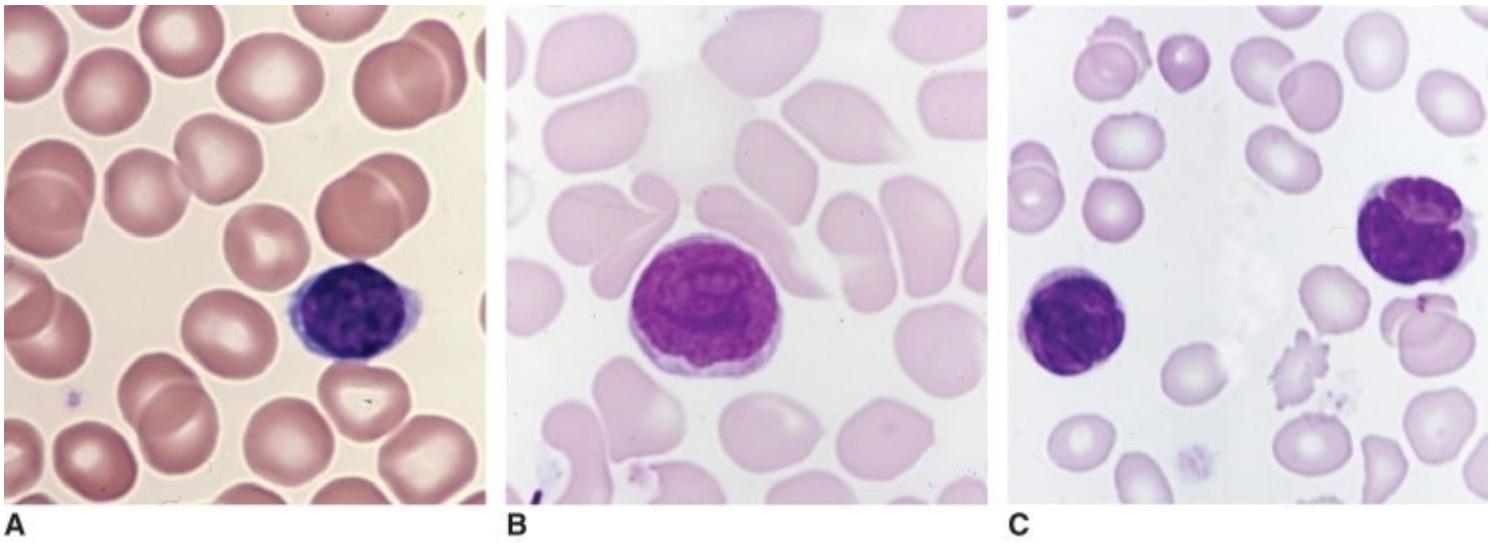


FIGURE 65–2 Blood lymphocytes. **A.** Normal small lymphocyte. **B.** Sézary cell. Note the nuclear swirls and the light microscopic appearance of the Sézary cell nucleus. Without careful inspection in cases of lymphocytosis, Sézary cells can be mistaken for small lymphocytes seen in chronic lymphocytic leukemia. **C.** Blood lymphocytes from a patient with mycosis fungoides and disseminated disease involving marrow and blood. Note clefted appearance of the nucleus. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

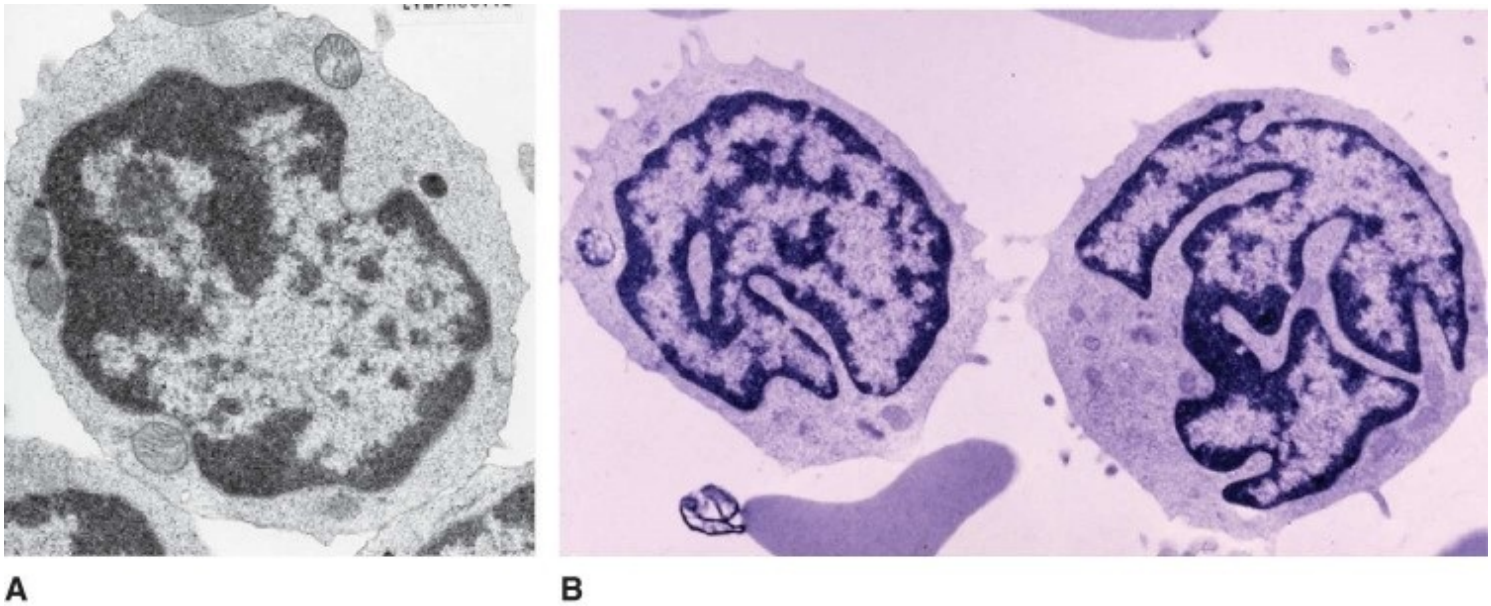


FIGURE 65–3 Transmission electron micrographs of lymphocytes. **A.** Normal lymphocyte. **B.** Two lymphocytes from a patient with Sézary cells in the blood. The latter have the striking cerebriform nuclear abnormalities characteristic of Sézary cells. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

LABORATORY FINDINGS

Skin Biopsy

- Early in the disease, skin biopsy may be compatible with several benign dermatoses.
- Classic mycosis fungoides lesions show a superficial lymphocytic infiltrate: lymphocytes may be large or small but have characteristic cerebriform nuclear convolutions.
  - — Epidermotropism is represented by clusters of lymphocytes in the epidermis around Langerhans cells referred to as Pautrier abscesses.
  - — Later in the disease, large lymphocytes may extend into the dermis.

Immunophenotype

- The neoplastic cells are mature helper-inducer T-cells expressing CD3, CD4, and CD45RO but not CD8. CD7, expressed on normal blood T cells, may be absent on mycosis fungoides lymphocytes in the skin and blood and on Sézary cells. Loss of CD26 expression is a hallmark of the neoplastic lymphocytes.

Lymph Node Biopsy

- Enlarged lymph nodes should have an excisional biopsy regardless of the tumor stage.
- Most early cases do not show effacement of node, but atypical lymphocytes in the paracortical T-cell zone are present.
- Later, lymph nodes may show partial or complete effacement and a monomorphic infiltrate of mycosis fungoides cells.

Chromosomal and Genic Findings

- The neoplastic lymphocytes have *TCRV*β gene rearrangement.
- Cytogenetic findings are not seen consistently. Loss of 10q heterozygosity and microsatellite instability are present in advanced disease. Microsatellite instability is a condition in which damaged DNA results from defects in normal DNA repair. Microsatellites (sections of DNA), which consist of a sequence of repeating units of one to six base pairs in length, either shorten or lengthen when unstable.
- Homozygous deletions of tumor suppressor genes *PTEN* and *CDKN2A* on chromosomes 9p and 10p are associated with progressive disease.

STAGING

- Mycosis fungoides is stratified according to the *tumor, node, metastasis, blood (TNMB) classification* (**Table 65–3**).
- Staging is of importance because it determines the therapeutic approach.
- Cutaneous lesions are stratified using the *T staging system* (**Table 65–3**).
- The presence of tumors (stage T3) may indicate a worse prognosis than erythroderma (stage T4).
- Lymph nodes are assigned the *N category* in the classification (**Table 65–3**).
- Superficial adenopathy, although present in many patients, is usually not prominent early in the disease but progresses with progressive skin involvement.
- The M stage (metastatic disease) is the most significant prognostic indicator (**Table 65–3**).
- Patients with liver, spleen, pleural, and lung involvement have a median survival of less than 1 year.
- Blood involvement is categorized in the *B* category in the classification system (**Table 65–3**).
- The percentage of neoplastic T cells in blood increases with progressive disease, but sensitive techniques can find small concentrations of neoplastic T cells in the blood at diagnosis in many patients.
- In most cases, lymph node architecture is not effaced, but dermatopathic changes are present with atypical lymphocytes in the T-cell paracortical areas. The latter finding carries

prognostic significance (Table 65–4).

TABLE 65–3 TNMB CLASSIFICATION OF MYCOSIS FUNGOIDES

T: Skin
- T1: Limited patches, papules, or plaques covering < 10% of the skin surface (T1a = patch only; T1b = plaques ± patches)
- T2: Generalized patches, papules, or plaques covering 10% of the skin surface (T2a = patch only; T2b = plaques ± patches)
- T3: At least one tumor (≥ 1 cm in diameter)
- T4: Generalized erythroderma over at least 80% body surface area

N: Lymph nodes
- N0: No clinically abnormal peripheral lymph nodes; biopsy not required
- N1: Clinically abnormal peripheral lymph nodes; histopathology Dutch grade 1 or NCI LN0 to 2
- N2: Clinically abnormal peripheral lymph nodes; histopathology Dutch grade 2 or NCI LN3
- N3: Clinically abnormal peripheral lymph nodes; histopathology Dutch grades 3–4 or NCI LN4
- NX: Clinically abnormal peripheral lymph nodes; no histologic confirmation

M: Visceral organs
- M0: No visceral organ involvement
- M1: Visceral organ involvement; requires histologic confirmation and specify organ

B: Blood
- B0: Atypical circulating cells not present (< 5%); specify "a" if flow cytometry is negative for clonal T lymphocytes or "b" if positive for clonal T lymphocytes
- B1: Atypical circulating cells present (> 5%, minimal blood involvement); specify "a" if flow cytometry is negative for clonal T lymphocytes or "b" if positive for clonal T lymphocytes
- B2: Leukemia (≥ 1.0×10^9 cells/L, CD4 to CD8 ratio of 10 or higher, evidence of a T-cell clone in the blood)

Note: T indicates the size of the tumor and whether it has invaded nearby tissue. N indicates the regional lymph nodes that are involved. M indicates distant metastasis. B indicates whether there are tumor cells in the blood.

Source: *Williams Hematology,* 9th ed, Chap. 103, Table 103–2.

TABLE 65–4 REVISED STAGING OF MYCOSIS FUNGOIDES AND SÉZARY SYNDROMES

	T	N	M	B
IA	1	0	0	0, 1
IB	2	0	0	0, 1
IIA	1, 2	1, 2	0	0, 1
IIB	3	0–2	0	0, 1
III	4	0–2	0	0, 1
IIIA	4	0–2	0	0
IIIB	4	0–2	0	1
IVA1	1–4	0–2	0	2
IVA2	1–4	3	0	0–2
IVB	1–4	0–3	1	0–2

See Table 65–3 for definitions of T1 to T4, N0 to N3, and M0 to M1.

Source: *Williams Hematology,* 9th ed, Chap. 103, Table 103–3.

DIFFERENTIAL DIAGNOSIS

- Several benign dermatoses can mimic mycosis fungoides and may even have *TCR* gene rearrangements. Examples are psoriasis and psoriasiform dermatoses (eczema, pityriasis

rubra pilaris, drug eruptions, and others).

- Adult T-cell leukemia-lymphoma may have skin lesions simulating mycosis fungoides.
  — Patients have other distinctive clinical features described in Chap. 66, including antibodies to the human T-cell lymphocytotropic virus (HTLV1).
- Pagetoid reticulosis (Woringer-Kolopp disease) consists of cutaneous plaques. It affects young males and has a benign course.
- Primary cutaneous CD30-positive lymphomas may have tumors that mimic mycosis fungoides. This is an indolent disease and may regress spontaneously. It should be distinguished from CD30+ transformation of mycosis fungoides and secondary skin involvement of CD30+ nodal lymphoma.
- Lymphoid papulosis is a benign skin disorder characterized by crops of pruritic, painful erythematous papules or nodules that ulcerate and heal spontaneously.

TREATMENT

- Treatment is divided into skin-directed and systemic therapy.
- Skin-directed therapy is the mainstay in early phases of disease and as an adjunct for systemic disease.
- Therapeutic options for mycosis fungoides and Sézary syndrome are listed in **Table 65–5**.
- Therapeutic modalities produce remission in most patients, but cure is uncommon.
- Topical glucocorticoids may be useful for pruritus, but they should not be used for long periods because they inhibit collagen synthesis and predispose to cutaneous infection. They should not be used on face, neck, or intertriginous areas. They can foster acne, glaucoma, and cataracts.
- Topical nitrogen mustard for early cutaneous disease has low toxicity but is not curative. It is also inconvenient to use as it must be applied daily to large areas of skin and frequent allergic responses occur. In responders, treatment should be continued for a year (or until lesions disappear) and then can be decreased in frequency for an additional year or two.
- Topical retinoids (eg, bexarotene) can induce complete responses in 20% and improvement in an additional 40%. They are approved for use in patients refractory to another topical therapy.
  — Retinoids must not be used in pregnant women.
- Phototherapy with ultraviolet (UV) radiation in the form of UVA or UVB spectrum can be useful in early disease, patches, and very thin plaques.
  — This therapy is given at least three times per week for 4 to 8 weeks to achieve maximal response.
  — It may result in complete clearing of lesions.
  — Acute cutaneous burning can occur and slight increase in long-term risk of other skin cancers.
- Psoralen with ultraviolet A light (PUVA) involves a psoralen dose of 0.6 mg/kg, orally, 2 hours before ultraviolet A light therapy, three times per week, followed by maintenance therapy given every 2 to 4 weeks indefinitely.
- PUVA results in 60% complete remission rate for patients with cutaneous plaques but, lower response rates for patients with generalized erythroderma or tumors.
  — Adverse effects include mild nausea, pruritus, and sunburn-like changes.

— PUVA is not curative.

- Electron-beam therapy is associated with a 80% complete remission rate; patients are 20% disease-free at 3 years.
  — This therapy involves 4 Gy/wk (total dose 36 Gy in 9 weeks).
  — It can be given to specific lesions or to the total skin surface.
- Oral retinoids (eg, bexarotene) 300 mg/m^2 per day can induce response in about 50% of patients and a complete response in about 2% of patients.
  — Virtually all patients develop central hypothyroidism and hypertriglyceridemia, which requires treatment with thyroid replacement and lipid-lowering agents.
  — Headaches, leukopenia, and pruritus may occur.
  — Retinoids are usually used in more advanced stages.
  — These agents must not be given to pregnant women or those considering pregnancy.
- Histone deacetylase inhibitors (eg, vorinostat and romidepsin), interferon-α, and a variety of single-agent chemotherapy (eg, pralatrexate, cyclophosphamide, fludarabine, doxorubicin) have been used.
  — Single-agent chemotherapy is occasionally effective but duration of response has been short, usually.
- Combined agent chemotherapy, also, has been used.
  — Combined agent therapy is more toxic, about 25% of patients have a good response but long-term disease-free survival is extremely uncommon.
- Other therapies, including monoclonal antibodies and antibody conjugates, recombinant fusion proteins, and extracorporeal photopheresis have been used.
- **Figure 65–4** illustrates the choice of treatment options related to the stage of disease.

TABLE 65–5 THERAPEUTIC OPTION FOR MYCOSIS FUNGOIDES AND SÉZARY SYNDROME

Skin-Directed Therapy	Systemic Therapy
Topical therapy Topical glucocorticoids Nitrogen mustard (mechlorethamine) Carmustine (BCNU, nitrosourea) Retinoids (bexarotene, tretinoin) Topical tacrolimus (Protopic) Imiquimod (Aldara)	Immunomodulators Interferon-α Extracorporeal photopheresis (ECP) Antibodies/fusion proteins Denileukin diftitox (ONTAK, DAB_{389}–IL-2) Alemtuzumab (Campath)
Light therapy UVB and PUVA Photodynamic therapy Electron beam Localized Total-skin	Retinoids Oral bexarotene (Targretin) Acitretin (Soriatane) Isotretinoin (Accutane) Histone deacetylase inhibitors Vorinostat (Zolinza) Romidepsin (Istodax) Chemotherapy (alone or in combinations) Oral prednisone, methotrexate, doxorubicin, cyclophosphamide, chlorambucil, pentostatin, cladribine, fludarabine, pralatrexate, several others

PUVA, psoralen and ultraviolet radiation of the A spectrum; UVB, ultraviolet radiation of the B spectrum.
Source: *Williams Hematology,* 9th ed, Chap. 103, Table 103–5.

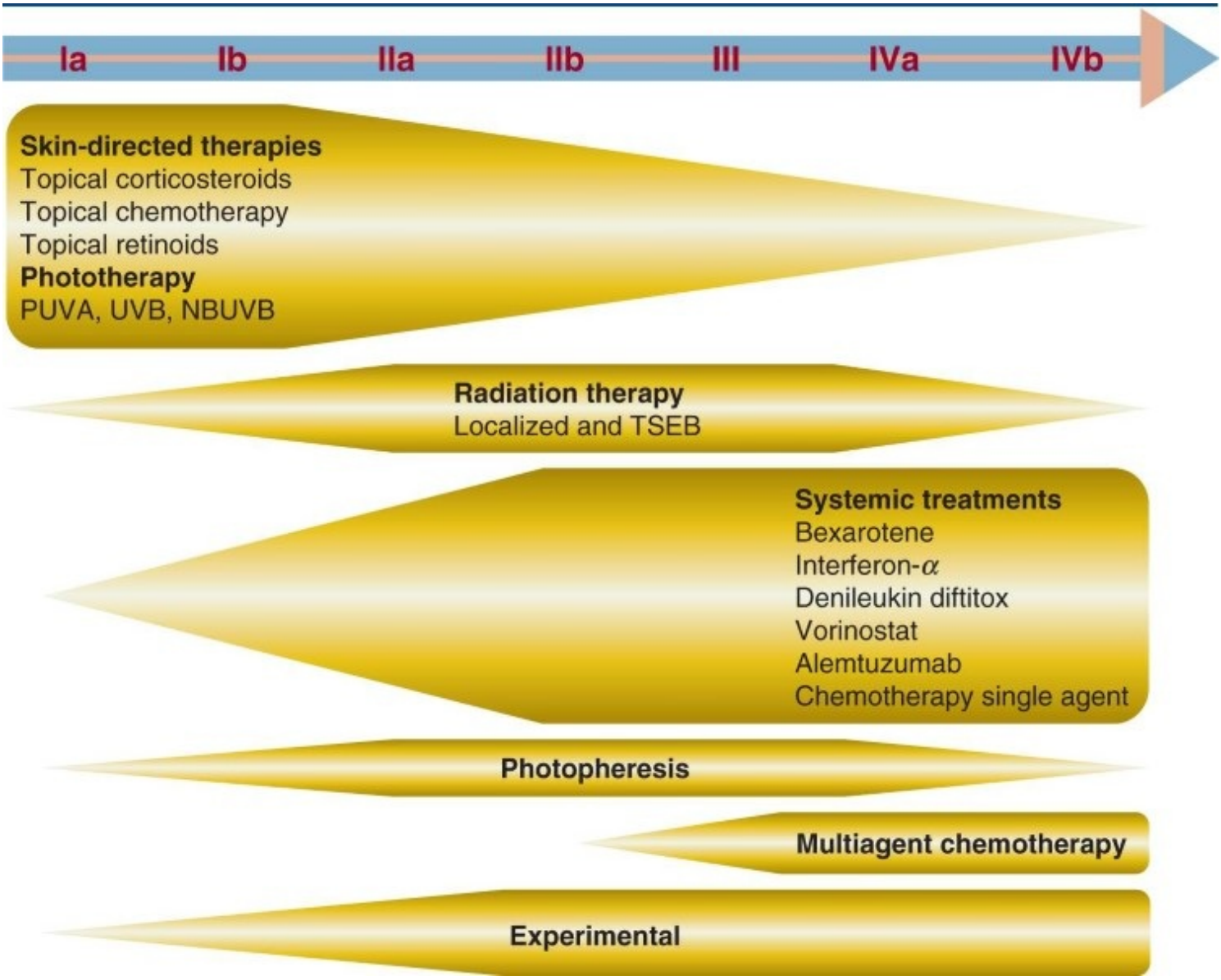


FIGURE 65–4 Cutaneous T-cell lymphoma treatment algorithm. NBUVB, narrowband ultraviolet B; PUVA, psoralen ultraviolet A; TSEB, total-skin electron beam; UVB, ultraviolet B. (Source: *Williams Hematology*, 9th ed, Chap. 103, Fig. 103–6.)

COURSE

- Median survival after diagnosis is about 12 years.
- Prognosis is dependent on the stage.
- Lymph node involvement signifies a poorer prognosis, and visceral involvement indicates poorest prognosis with median survival of less than 1 year.
- Fifty percent of deaths in patients with mycosis fungoides result from infection.
- Septicemia and bacterial pneumonia are common. *Pseudomonas* sp and *Staphylococcus* sp are particularly common and originate in the skin.
- Herpes virus infection occurs in 10% of patients.

PRIMARY CUTANEOUS ANAPLASTIC LARGE CELL LYMPHOMA

- CD30+ cutaneous lymphoproliferative disease is the second most common CTCL after

mycosis fungoides and represents approximately 25% of cutaneous T-cell disorders.

- This disorder represents a spectrum from lymphomatoid papulosis, a benign self-limited form, to cutaneous anaplastic large cell lymphoma (ALCL), a more progressive form.
- CD30+ primary cutaneous ALCL presents with skin involvement without evidence of extracutaneous disease for at least 6 months after presentation (**Figure 65–5**).
- CD30+ primary cutaneous ALCL can occur at any age. The median incidence is about 65 years and males are affected slightly more often than females.
- The lesions are brownish to violaceous nodules or tumors. Often solitary, they can be numerous and widely distributed and may regress spontaneously.
- On biopsy, at least 75% of the cells should express CD30 and usually CD4 but are negative for CD15, do not express ALK-1, or have the t(2;5), unlike systemic ALCL.
- For localized disease, radiotherapy is usually utilized.

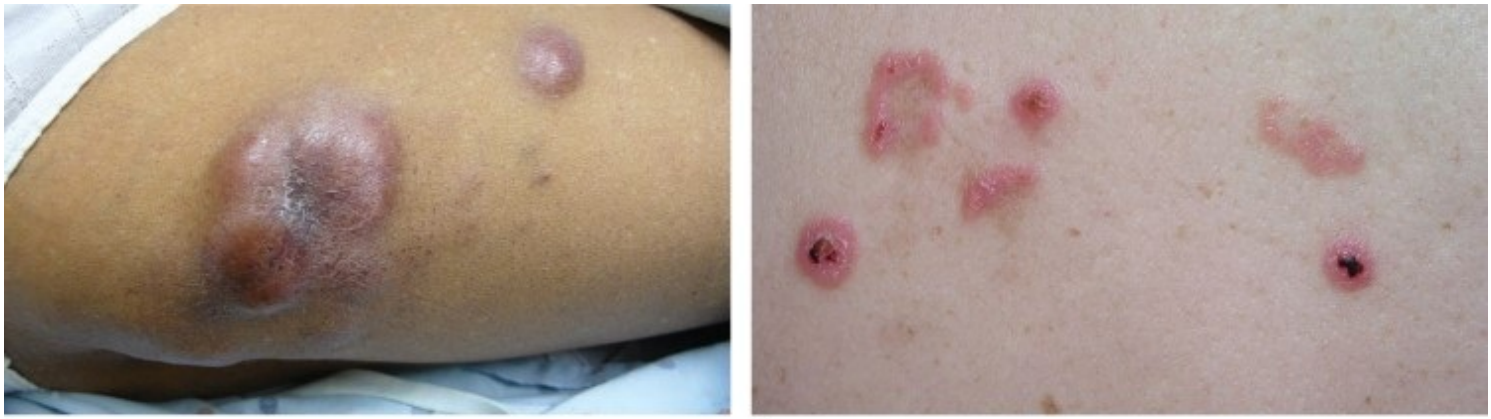
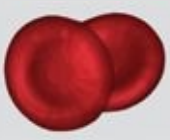


FIGURE 65–5 CD30+ lymphoproliferative disorders. **A.** Primary cutaneous anaplastic large cells lymphoma. Large cutaneous tumors on the anterior thigh. **B.** Lymphomatoid papulosis. Numerous small erythematous papules and small nodules. Some with necrotic centers in crops. Some lesions show spontaneous regression. (Source: *Williams Hematology,* 9th ed, Chap. 103, Fig. 103–7.)

LYMPHOID PAPULOSIS

- Lymphoid papulosis is a clonal, usually self-limited disease characterized by crops of erythematous, dome-shaped papules or nodules that may ulcerate. They regress over a few months with minor residuals of scarring or atrophy.
- Lymphoid papulosis, rarely, can evolve into a more aggressive cutaneous lymphoma. It is also associated with a higher incidence of lymphoid and nonlymphoid malignancies than unaffected persons.
- Observation without specific therapy is often the best management. Other treatments can include low-dose methotrexate, brentuximab vedotin, and radiotherapy.



For a more detailed discussion, see Larisa J. Geskin: Cutaneous T-Cell Lymphoma (Mycosis Fungoides and Sézary Syndrome), Chap. 103 in *Williams Hematology,* 9th ed.

CHAPTER 66
Mature T-Cell and Natural Killer Cell Lymphomas

- The term *peripheral T-cell lymphoma* (PTCL) refers to lymphomas originating in a mature (ie, post-thymic) T-cell.
- PTCLs make up 10% to 15% of all non-Hodgkin lymphomas and represent at least 23 heterogeneous diseases.
- **Table 66–1** lists the most common of the mature T-cell and natural killer (NK) cell lymphomas.
- The incidence of various types of PTCL varies widely based on geography (**Table 66–2**).

TABLE 66–1 **2008 WHO CLASSIFICATION OF MATURE T-CELL AND NATURAL KILLER-CELL NEOPLASMS (EXCLUDING PRIMARY CUTANEOUS LYMPHOMA)**

Peripheral T-cell lymphoma, NOS
Angioimmunoblastic T-cell lymphoma
Anaplastic large cell lymphoma, ALK-positive
Anaplastic large cell lymphoma, ALK-negative
Enteropathy-associated T-cell lymphoma
Adult T-cell leukemia/lymphoma
Hydroa vacciniforme–like lymphoma
T-cell prolymphocytic leukemia
T-cell large granular lymphocytic leukemia
Hepatosplenic T-cell lymphoma
Extranodal NK/T-cell lymphoma, nasal type
Aggressive NK cell leukemia
Systemic EBV+ T-cell lymphoproliferative disease of childhood (associated with chronic active EBV infection)
Chronic lymphoproliferative disorder of NK cells*

ALK, anaplastic lymphoma kinase; EBV, Epstein-Barr virus; NK, natural killer; NOS, not otherwise specified.
*Provisional entity.
Source: *Williams Hematology*, 9th ed, Chap. 104, Table 104–1.

TABLE 66–2 **INCIDENCE OF LYMPHOMA SUBTYPES BY GEOGRAPHIC REGION**

Subtype	Registry	PTCL-NOS	AITL	ALCL ALK+	ALCL ALK–	NK/T	ATL	EATL
North America	IPTCL	34%	16%	16%	8%	5%	2%	6%
	BCCA	59%	5%	6%	9%	9%	NA*	5%
	COMPLETE	34%	15%	11%	8%	6%	2%	3%
Europe	IPTCL	34%	29%	6%	9%	4%	1%	9%
	Swedish	34%	14%	9%	15%	4%	NA*	9%
Asia	IPTCL	22%	18%	3%	3%	22%	25%	2%

AITL, angioimmunoblastic T-cell lymphoma; ALCL ALK–, anaplastic large cell lymphoma anaplastic lymphoma kinase negative; ALCL ALK+, anaplastic large cell lymphoma anaplastic lymphoma kinase positive; ATLL, adult T-cell leukemia/lymphoma; BCCA, British Columbia Cancer Agency; COMPLETE, Comprehensive Oncology Measures for Peripheral T-Cell Lymphoma

Treatment; EATL, enteropathy-associated T-cell lymphoma; IPTCL, International Peripheral T-Cell Lymphoma Project; NA, not available; NK/T, natural killer–cell/T-cell lymphoma; NOS, not otherwise specified; PTCL, peripheral T-cell lymphoma.
*ATLL patients were excluded in both the BCCA and Swedish Registry Studies.
Source: *Williams Hematology*, 9th ed, Chap. 104, Table 104–2.

PERIPHERAL T-CELL LYMPHOMA

- The diagnosis of PTCL is based on histologic features, immunophenotype, molecular studies, and clinical presentation.
- B-cell lymphomas are characterized by immunophenotypic features, whereas T-cell lymphomas are characterized by antigen aberrancy, which may vary within a subtype and over the course of the disease.
- Pathologists have a low concordance rate when reviewing the histopathology of a PTCL as compared to a mature B-cell lymphoma.
- In the diagnosis of PTCL it is important to exclude a reactive process, particularly when the clinical picture is not congruent with the pathological features, when the diagnostic biopsy is small, or when a clonal T-cell receptor rearrangement is the only reason for the diagnosis.

Diagnostic Evaluation

- Initial evaluation should include a history and physical examination; computed tomography (CT) of the chest, abdomen, and pelvis or positron emission tomography (PET)/CT imaging of those areas; a marrow aspirate and biopsy; and laboratory evaluation to include complete blood cell count, serum lactic dehydrogenase (LDH), a metabolic panel, and serological testing for human T-cell lymphocytotropic virus-1(HTLV-1) in patients from endemic areas.
- The International Prognostic Index (IPI) is useful in stratification of patients with peripheral T-cell lymphomas except in the case of angioimmunoblastic T-cell lymphoma.
  — The IPI considers age, performance status, serum LDH level, and marrow involvement (**Table 66–3**).
- Gene expression profiling has identified molecular features that improve classification and prognostication of anaplastic lymphoma kinase (ALK)-negative anaplastic large cell lymphoma (ALCL), angioimmunoblastic T-cell lymphoma (AITL), and PTCL not otherwise specified (PTCL-NOS).
  — For example a t(6;7) identifies a unique entity within ALK-negative ALCL with a favorable prognosis.

TABLE 66–3 CHARACTERISTICS AND OUTCOMES IN COMMON PERIPHERAL T-CELL LYMPHOMA SUBTYPES

PTCL Subtype	Number	Median Age	% IPI			5-Year OS*	5-Year PFS*	5-Year OS by IPI	
			0–1	2–3	4–5			0–1	4–5
PTCL-NOS									
IPTCL	229	60	28	57	15	32%	20%	50%	11%
BCCA	117	64	30	47	22	35%	29%	64%	22%
Swedish	256	69	17†	59†	24†	28%	21%	NA	NA

AITL									
IPTCL	213	65	14	59	28	32%	18%	56%	25%
BCCA	10	66	0	30	70	36%	13%	NA	NA
Swedish	104	70	4[†]	69[†]	27[†]	31%	20%	NA	NR
ALCL ALK–									
IPTCL	72	58	41	44	15	49%	36%	74%	13%
BCCA	18	55	44	22	33	34%	28%[‡]	66%[‡]	25%[‡]
Swedish	115	67	34	42	24	38%	31%	NA	NA
ALCL ALK+									
IPTCL	76	34	49	37	14	70%	60%	90%	33%
BCCA	12	32	67	25	8	58%	28%[‡]	66%[‡]	25%[‡]
Swedish	68	41	55[†]	39[†]	6[†]	79%	63%	NA	NA
EATL									
IPTCL	62	61	25	63	13	20%	4%	29%	15%
BCCA	9	61	0	30	70	22%	22%	NA	NA
Swedish	68	68	42	44	14	20%	18%	NA	NA
NK/T IPTCL									
Extranasal	35	44	26	57	17	9%	6%	17%	20%
Nasal	92	52	51	47	2	42%	29%	57%	0%
BCCA	17	47	47	24	29	24%	15%	38%	20%
Swedish	33	62	33	63	4	21%	14%	NA	NA

AITL, angioimmunoblastic T-cell lymphoma; ALCL ALK–, anaplastic large cell lymphoma anaplastic lymphoma kinase negative; ALCL ALK+, anaplastic large cell lymphoma anaplastic lymphoma kinase positive; BCCA, British Columbia Cancer Agency; EATL, enteropathy-associated T-cell lymphoma; IPI, International Prognostic Index; IPTCL, International Peripheral T-Cell Lymphoma Project; NA, not available; NK/T, natural killer cell/T-cell lymphoma; NOS, not otherwise specified; OS, overall survival; PFS, progression-free survival; PTCL, peripheral T-cell lymphoma.

*Data from International T-cell lymphoma project in which >85% of patients received an anthracycline-based regimen without upfront transplant.

[†]Distribution of patients with the given IPI scores is based on the number of patients for whom the score could be completely calculated.

[‡]BCCA ALCL reported as both ALK + and –.

Source: *Williams Hematology*, 9th ed, Chap. 104, Table 104–3.

Initial Treatment

- **C**yclophosphamide, **h**ydroxydaunorubicin (doxorubicin), vincristine (**O**ncovin), **p**rednisone (CHOP) is the most widely used therapy for patients with PTCL.
  - — Only patients with anaplastic large cell lymphoma whose tumors overexpress ALK have a 5-year failure-free survival of more than 50%.
- Several regimens have been devised in an attempt to improve the outcome of patients with PTCL. These include adding the anti-CD52 antibody, alemtuzumab, to CHOP, adding etoposide to CHOP, or adding autologous hematopoietic stem cell transplantation for patients in first remission after chemotherapy.
  - — Some favor adding etoposide to CHOP for patients who are younger than 60 years of age.
  - — Patients older than 60 years of age or with significant comorbidities have increased toxic effects from adding etoposide.

Approach to Relapsed or Refractory PTCL

- There are no randomized trials to guide treatment of patients with relapsed or refractory PTCL.
- Four agents—pralatrexate, romidepsin, belinostat, and brentuximab vedotin—are approved for the treatment of patients with relapsed PTCL.
- Brentuximab vedotin is approved for patients with anaplastic large cell lymphoma if the tumor expresses CD30 (**Table 66–4**).
- Both autologous and allogeneic hematopoietic stem cell transplantation have been utilized in patients with relapsed/refractory PTCL and some patients have prolonged failure-free survival.

TABLE 66–4 **OVERALL RESPONSE RATE TO AGENTS FDA APPROVED FOR RELAPSED/REFRACTORY PERIPHERAL T-CELL LYMPHOMA**

Subtype	Pralatrexate	Romidepsin	Belinostat	Brentuximab Vedotin
PTCL-NOS	31%	29%	23%	33%
AITL	8%	30%	46%	50%
ALCL	29%	24%	15%	86%

AITL, angioimmunoblastic T-cell lymphoma; ALCL, anaplastic large cell lymphoma; PTCL, peripheral T-cell lymphoma; PTCL-NOS, peripheral T-cell lymphoma, not otherwise specified.
Source: *Williams Hematology*, 9th ed, Chap. 104, Table 104–4.

Specific Subtypes of PTCL

Peripheral T-Cell Lymphoma Not Otherwise Specified

- PTCL-NOS does not fit into another recognized category.
- These PTCLs are the most common of the categories of PTCL, making up approximately 25% of the total cases in Western countries.
- Median age is 60 years with a male predominance.
- This is an aggressive lymphoma with frequent constitutional symptoms, extranodal involvement, and elevated serum LDH; approximately 70% of the patients are stage III or IV.
- Most tumors express CD4, although a minority has CD8 expression or combined CD4 and CD8 expression.
- The addition of etoposide to CHOP seems to provide some benefit in young patients. In a prospective study of etoposide plus CHOP followed by autologous hematopoietic stem cell transplantation, the overall response rate was 82%, with 51% of the patients achieving a complete remission.
- In one large study, 38% of the patients with PTCL-NOS who had an autologous hematopoietic stem cell transplant in remission had a 5-year failure-free survival.
- Allogeneic hematopoietic stem cell transplantation can provide durable remissions in a subset of patients with relapsed PTCL-NOS.
- Pralatrexate, romidepsin, and belinostat have an approximate response rate of 25% in patients with relapsed PTCL-NOS.

Angioimmunoblastic T-Cell Lymphoma

- AITL represents approximately 20% of all PTCLs and is more frequent in Europe than other

areas.

- The male-to-female ratio is approximately 1:1 and the median age of diagnosis is approximately 70 years.
- Common features at presentation include fevers, drenching sweats, weight loss, generalized adenopathy, rash, polyclonal hypergammaglobulinemia, blood eosinophilia, and autoimmune hemolytic anemia.
- Some patients have waxing and waning of symptoms over several years before a diagnosis is made.
- Patients with AITL can develop a diffuse large B-cell lymphoma in the T-cell lymphoma, which evolves from Epstein-Barr virus (EBV)–positive B-cells that are usually present in the lymphoid infiltrate.
- The normal counterpart of the cell of origin of AITL is thought to be the follicular T-helper cell.
- Patients with AITL treated with CHOP have an outcome similar to that seen in PTCL-NOS.
- Rare patients may be managed with glucocorticoid monotherapy, although the responses are rarely sustained.
- Responses to low-dose methotrexate and cyclosporine have been reported.
- Patients with CD30-positive AITL can benefit from brentuximab vedotin.

Anaplastic Large Cell Lymphoma

- The prevalence of ALCL varies by geographic region (see **Table 66–2**).
- Younger patients with ALCL usually overexpress the ALK protein (90% of children are ALK-positive). The median age of patients who develop ALK-positive ALCL is 35 years and the median age of patients with ALK-negative ALCL is 58 years.
- ALCL has an aggressive clinical course with frequent systemic symptoms (fever, night sweats, weight loss) and advanced distribution of disease at presentation.
- A rare clinical variant of ALK-negative ALCL is associated with saline and silicone breast implants. The natural history of this subtype seems to be less aggressive and patients with localized disease may be adequately treated by surgical removal of the implant and the capsule.
- ALCL cells tend to grow cohesively and are found preferentially invading lymph nodes sinuses.
- ALK-positive ALCL is characterized by a t(2;5) (p32;q35) translocation involving fusion of the *NPM* and *ALK* gene. The resultant *NPM-ALK* fusion protein acts as an oncogene.
- ALK-positive ALCL is the most chemosensitive of the T-cell lymphomas with rates of survival and response similar to that of diffuse large B-cell lymphoma.
- ALK-positive ALCL is commonly seen in the pediatric age group and 65% of pediatric patients treated with an anthracycline-based chemotherapy regimen remain relapse-free after 5 years.
- Among adults, treatment with a CHOP regimen with or without added drugs remains the most commonly used approach.
- The IPI seems to be particularly helpful in risk stratification in ALK-positive ALCL.
- In one German study, patients who received CHOP plus etoposide for ALK-positive ALCL had a particularly good response.

- Brentuximab vedotin has an 80% response rate in patients with relapsed or refractory ALCL.
- Crizotinib, an inhibitor of the ALK tyrosine kinase, has demonstrated encouraging responses in ALK-positive ALCL.

Enteropathy-Associated T-Cell Lymphoma

- Enteropathy-associated intestinal T-cell lymphoma (EATL) is a mature T-cell lymphoma that presents in the gastrointestinal tract.
- EATL varies in incidence geographically.
- The median age at diagnosis is 60 years with a slight male predominance.
- EATL is divided into type I and type II.
  — Type I is most commonly seen in patients with underlying celiac disease, is strongly associated with a human leukocyte antigen (HLA-DQ2 haplotide), and comprises 6% to 80% of the cases.
  — Type II EATL is less frequently associated with sprue.
- The majority of patients with EATL present with acute abdominal symptoms that often require urgent surgery.
  — EATL frequently presents with ulcerative lesions in jejunum or ileum, which may perforate. Extraintestinal presentation of the EATL is rare.
- CHOP chemotherapy has been used most frequently with a 5-year freedom from relapse of 4% to 22% and a 5-year survival of approximately 20%.
- In patients who are eligible, high-dose chemotherapy followed by autologous hematopoietic stem cell transplant may improve outcomes.

Adult T-Cell Lymphoma Leukemia

- Adult T-cell lymphoma leukemia (ATL) is caused by infection with the retrovirus HTLV-1.
- Tumor cells circulating in the blood have a “flower-cell” nuclear appearance (**Figure 66–1**).
- The disease is rare in the United States but frequent in southern Japan, parts of Central and South America, tropical Africa, Romania, and northern Iran.
- Approximately 15 million HTLV-1 carriers exist. A lifetime risk of developing ATL is approximately 2.5% to 4% with a mean latency period of greater than 50 years.
- HTLV-1 is transmitted through breastfeeding, blood products, and unprotected sexual intercourse.
- The median age of patients with ATL is 62 years without gender predominance.
- Clinical variants include acute, lymphoma, chronic, and smoldering types.
- The acute variant represents 6% of cases and is characterized by patients who present with a frank leukemic presentation.
- An additional 20% of cases present with the lymphoma variant characterized by lymphadenopathy and less than 1% leukemic cells in the blood.
- Hepatosplenomegaly, elevated serum LDH, hypercalcemia, and skin lesions are frequent.
- The leukemic and lymphoma subtypes follow an aggressive clinical course with a median survival of less than 1 year.
- The prognosis of the aggressive forms of ATL remains poor. Multidrug chemotherapy regimens are utilized, but durable remissions are rare.

- The smoldering and chronic forms follow an indolent course with a median survival of approximately 4 years.
- The role of antiviral therapy remains controversial.
- The monoclonal antibody mogamulizumab (an anti-CCR4 monoclonal antibody) had a response rate of 50% and a median survival of 13 months in relapsed/refractory patients.
- Allogeneic hematopoietic stem cell transplantation in a large Japanese series showed a 3-year overall survival of 33%.

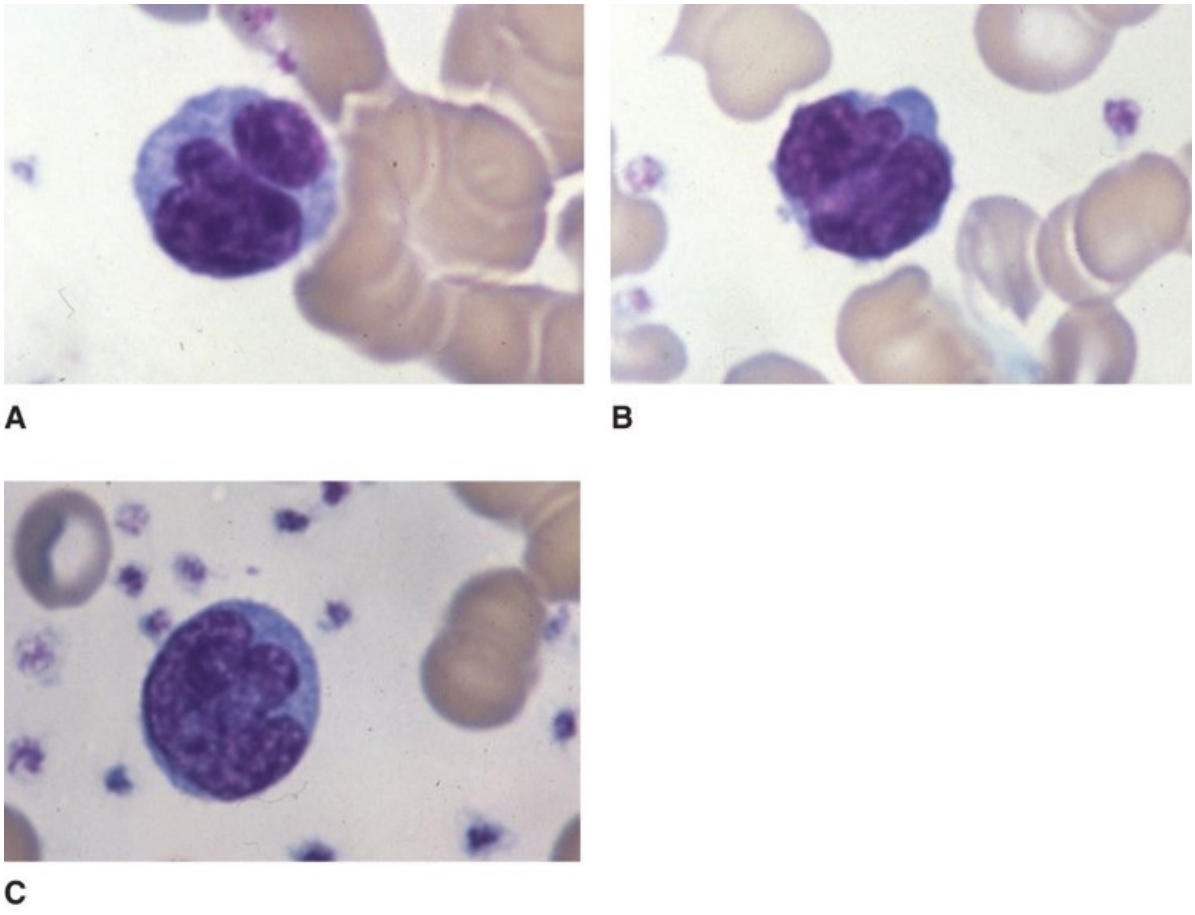


FIGURE 66–1 Blood film from a patient from the Caribbean region with adult T-cell leukemia-lymphoma. **A–C.** Note highly lobulated and clefted nuclei in lymphocytes, findings characteristic of this disease. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

Hepatosplenic T-Cell Lymphoma

- Hepatosplenic T-cell lymphoma (HSTCL) is a rare lymphoma that involves the spleen, liver, and marrow.
- In the majority of cases, the tumor cells consist of mature gamma-delta T-cells. However, an alpha-beta T-cell subset has been reported.
- The disease typically occurs in young males with a median age of 35 years. It is frequently seen in patients undergoing immunosuppression following organ transplantation or following the use of anti–tumor necrosis factor alpha agents for inflammatory bowel disease or other autoimmune diseases.
- Patients usually present with isolated hepatosplenomegaly, without lymphadenopathy, and with cytopenias, B symptoms, and elevated serum LDH.
- Neoplastic cells are localized to the sinuses in the spleen, liver, and marrow.

- HSTCL follows an aggressive clinical course, and it has a median survival of 16 months.
- The outcome with CHOP therapy is poor and some suggest a better outcome with other non–cross-resistant regimens such as ifosfamide, carboplatin, etoposide (ICE); cyclophosphamide, vincristine, doxorubicin (Adriamycin), dexamethasone, methotrexate, cytarabine (hyper-CVAD); or ifosfamide, VP-16 (etoposide), amethopterin (methotrexate), cytarabine (IVAC).
- Successful treatment with pentostatin has been reported.
- Consolidation with either allogeneic or autologous hematopoietic stem cell transplantation is usually necessary for long-term remission.

Extranodal NK/T-Cell Lymphoma

- Extranodal NK/T-cell lymphoma (ENKTL), nasal type, was previously known as lethal midline granuloma.
- ENKTL represents approximately 5% of T-cell lymphomas.
- The disease typically affects middle-aged men at a median age of 50 years.
- ENKTL occurs worldwide with a strong geographic predilection for Asian people from China, Japan, Korea, and Southeast Asia, and for Central and South American people from Mexico, Peru, Argentina, and Brazil.
- ENKTL is almost exclusive extranodal—most commonly involving the nose, nasopharynx and paranasal sinuses in addition to tonsils, Waldeyer ring, and oropharynx.
- Although these tumors are usually localized, they can disseminate to other organs, including the gastrointestinal tract and skin.
- The tumor can occasionally present in extranodal sites.
- Histopathology shows pleomorphic small-to-medium atypical lymphoid cells with vascular invasion and ischemic tissue necrosis.
- Treatment outcome of localized ENKTL is best achieved with combined chemotherapy and radiation therapy.
- In studies of radiation therapy alone, 75% to 100% of the patients respond. However, the systemic relapse rate is as high at 35%.
- Patients treated with CHOP-based therapy and radiation have a complete remission rate of 60% and 3-year disease free survival of 25%.
- Asparaginase is a very active agent in this disease. Asparaginase combined with gemcitabine, oxaliplatin, and radiotherapy exhibits a response rate of 96% and a relapse rate of 10% to 15%.
- An intensive chemotherapy regimen, SMILE (dexamethasone, methotrexate, ifosfamide, L-asparaginase and etoposide), in combination with radiotherapy demonstrates a complete remission rate of 78%. The same regimen has demonstrated response rates of 25% to 80% in disseminated disease.
- A prognostic index has been developed for patients with ENKTL, including B-symptoms, elevated serum LDH, disease stage, and regional lymphadenopathy. The 5-year survival of patients with none, one, two, or three or more of these risk factors was 81%, 64%, 34%, and 4% respectively.
- NK-cell leukemia is an extremely aggressive subtype of T-cell leukemia characterized by involvement in the blood and marrow and a survival measured in weeks.

NK-LARGE GRANULAR LYMPHOCYTIC CELL LEUKEMIA

- NK-large granular lymphocytic (LGL) cell leukemia is an extremely aggressive subtype of T-cell leukemia characterized by involvement in the blood and marrow and a survival measured in weeks.

Etiology and Pathogenesis

- EBV infection is implicated in the pathogenesis.

Clinical Features

- Fever, night sweats, weight loss are common.
- Massive hepatosplenomegaly is typical.
- Lymphadenopathy and gastrointestinal tract involvement are common.
- Patients tend to be of younger age than those with CD3+ LGL leukemia.

Laboratory Features

- LGL lacks expression of CD3 and clonal T-cell receptor rearrangements.
- NK-leukemic LGL cells usually have the immunophenotype CD4CD16CD56+CD8CD57–.
- LGL counts are generally high and may exceed 50×10^9/L.
- Anemia and thrombocytopenia very common.
- Severe neutropenia (eg, $<0.5 \times 10^9$/L) is observed in less than one-fifth of patients.
- Coagulopathy frequently occurs.
- Clonal cytogenetics may be present.

Treatment, Course, and Prognosis

- Acute presentation and an aggressive course is common.
- Patients with chronic NK lymphocytosis may not require treatment.
- Effective combination chemotherapy has not been reported.
- Patients with NK-LGL usually die a few months after diagnosis despite aggressive multidrug chemotherapy.

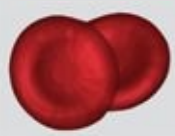
Subcutaneous Panniculitis-Like T-Cell Lymphoma

- This very uncommon form of lymphoma occurs in less than 1% of cases.
- The median age of presentation is 30 years, and 20% of patients are younger than age 20 years.
- About one-fifth of patients have an associated autoimmune disease, usually disseminated lupus erythematosus.
- Most common presentation is that of multiple, painful subcutaneous nodules on the trunk and extremities without other apparent sites of involvement. The nodules may range from 0.5 to several centimeters in diameter and may become necrotic.
- The lesions may regress, only to reappear later.
- Fever, night sweats, and weight loss are present in 50% of patients.
- Blood cytopenias may be present at diagnosis.

- The hemophagocytic syndrome may accompany this lymphoma in as many as 20% of patients.
- The cells have a mature αβ T-cell phenotype and usually are CD8+ and express granzyme B and perforin.
- Dissemination to lymph nodes is very unusual.
- The 5-year survival is approximately 80%. However, onset of the hemophagocytic syndrome is a very poor prognostic sign.
- Multidrug lymphoma therapy has been the mainstay of treatment, but studies indicate less intensive regimens (eg, chlorambucil, prednisone, cyclosporine) may be as useful.
- A distinction from cutaneous γδ T-cell lymphoma is important because the latter does not have as favorable a prognosis.

HYPEREOSINOPHILIA AND T-CELL LYMPHOMAS

- Blood eosinophilia of greater than 1×10^9/L occurs in approximately 20% of T-cell lymphomas, and about 20% of patients with the hypereosinophilic syndrome have a clonal T-cell expansion as the underlying cause.
- The neoplastic T-cells secrete interleukin (IL)-5, the principal eosinophilopoietin. Intratumoral IL-5 elaboration is closely correlated with blood eosinophilia.
- The clonal expansion of T cells, manifest principally by blood hypereosinophilia, progresses to overt and progressive T-cell lymphoma in more than 25% of patients over 10 years of observation.



For a more detailed discussion see Neha Mehta, Alison Moskowitz, and Steven Horwitz: Mature T-Cell and Natural Killer Cell Lymphomas, Chap. 104 in *Williams Hematology*, 9th ed.

CHAPTER 67
Essential Monoclonal Gammopathy

DEFINITION

- Essential monoclonal gammopathy is the presence of a serum monoclonal immunoglobulin (Ig) or a serum and urine monoclonal immunoglobulin light chain in the absence of evidence for a B-cell tumor (eg, B-cell lymphoma, macroglobulinemia, myeloma, plasmacytoma, amyloidosis) over a period of observation.
- The monoclonal immunoglobulin may be of any isotype and may occasionally be of multiple isotypes (see **Table 67–1**).
- Synonyms for essential monoclonal gammopathy include (1) monoclonal gammopathy; (2) benign monoclonal gammopathy; and (3) monoclonal gammopathy of unknown significance, with the acronym MGUS. The latter seems less appropriate now that the significance of this diagnosis is precisely known, and it is one of many examples of nonprogressive, clonal disorders with a predisposition to undergo clonal evolution to a malignant disorder (eg, adenomatous colonic polyp), making its significance apparent.

TABLE 67–1 **TYPES OF MONOCLONAL IMMUNOGLOBULIN SYNTHESIZED BY B-CELL CLONE IN ESSENTIAL MONOCLONAL GAMMOPATHY**

Serum IgG, IgA, IgM, IgE, or IgD
Serum IgG + IgA, IgG + IgM, IgG + IgA + IgM
Serum monoclonal κ or λ light chain*

*Urinary monoclonal immunoglobulin light chain excretion (Bence Jones proteinuria) may accompany serum monoclonal light chain.
Source: *Williams Hematology,* 9th ed, Chap. 106, Table 106–1.

EPIDEMIOLOGY

- Essential monoclonal gammopathy may occur at any age, but it is very unusual before puberty and increases in frequency with age. Frequency approximately is 1% in those over 25 years, 3% in those over 70 years, and 10% in those over 80 years of age based on zonal electrophoresis studies.
- Frequency is higher with more sensitive immunologic techniques (eg, isoelectric focusing or immunoblotting).
- Frequency is significantly greater among Americans of African descent than those of European descent in comparative age groups.
- Frequency is greater in males than females.
- Familial aggregation of persons with essential monoclonal gammopathy occurs.

- Essential monoclonal gammopathy may harbinger the future development of a B-cell neoplasm (eg, myeloma). Most, and perhaps, all patients with myeloma evolve from a preceding essential monoclonal gammopathy.

ETIOLOGY AND PATHOGENESIS

- The gammopathy results from the growth of a single mutated B-lymphocyte into a clone of cells that elaborate a monoclonal immunoglobulin and or monoclonal light chains. Cessation of expansion of the clone occurs and the size of the clone remains stable, at a steady-state of approximately 1 to 5×10^{10} cells, indefinitely.
- At this clone size, organ pathology such as osteolysis, hypercalcemia, renal disease, or hematopoietic suppression does not occur. Polyclonal immunoglobulin synthesis is usually normal and, thus, increased frequency of infections is not a feature.
- IgA and IgG monoclonal gammopathy arise from a somatically mutated postswitch preplasma cell, and IgM monoclonal gammopathy arises from a mutated germinal center B lymphocyte without evidence of isotype switching. This feature influences the result of clonal progression: IgA and IgG monoclonal gammopathy tend to evolve into myeloma or amyloidosis and IgM monoclonal gammopathy tends to progress to lymphoma or macroglobulinemia.
- The monoclonal immunoglobulin may react against self-antigen(s), resulting in symptomatic disease (eg, neuropathy) that depends on the self-antigen involved and its blood or tissue distribution (see **Table 67–2**).

TABLE 67–2 FUNCTIONAL ABNORMALITIES ASSOCIATED WITH ESSENTIAL MONOCLONAL GAMMOPATHY

Plasma protein disturbances
Antierythrocyte antibodies, acquired von Willebrand disease, immune neutropenia, cryoglobulinemia, cryofibrinogenemia, acquired C1 esterase inhibitor deficiency (angioedema), acquired antithrombin, insulin antibodies, antiacetylcholine receptor antibodies, "antiphospholipid" antibodies, dysfibrinogenemia
Renal disease
Oculopathies
Neuropathies
Deep venous thrombosis

Source: *Williams Hematology,* 9th ed, Chap. 106, Table 106–2.

CLINICAL FEATURES

- Persons with essential monoclonal gammopathy do not have symptoms or signs of myeloma or another B-cell lymphoproliferative disease (eg, anemia, marrow plasmacytosis, lymph node enlargement, plasmacytoma, bone lesions, or amyloid deposits).
- Individuals usually are detected by the unexpected identification of a monoclonal protein in plasma or urine by zonal protein electrophoresis or another technique (see "Laboratory Detection," below).
- Patients occasionally have features of diseases that may result from the interaction of the

monoclonal protein with antibody specificity for a plasma or cell protein (eg, acquired von Willebrand disease, neuropathy, others).

- Patients may also have symptomatic disease because of the physicochemical features of the monoclonal immunoglobulin, such as predisposition to form crystals (eg, corneal keratopathy, Fanconi renal disease) or exaggerated copper binding (pseudo–Kayser-Fleischer corneal rings) resulting in visual impairment.
- **Table 67–2** lists the clinical abnormalities or diseases that can occur as a result of this effect of the monoclonal protein.

LABORATORY DETECTION

Zonal Electrophoresis and Serum Light Chain Assay

- Serum protein electrophoresis and serum light chain assays are used to determine κ:λ light chain ratio.
- Molecules of each monoclonal protein have identical size and charge and thus migrate as a narrow band.
- Electrophoresis also can be done on concentrated samples of urine or cerebrospinal fluid (CSF).
- Immunoelectrophoresis and immunofixation electrophoresis are used to identify the heavy-chain class and light-chain type of monoclonal proteins.
- Serum and urine light chain assays may be useful.

LABORATORY FEATURES

- The monoclonal protein is usually IgG but may be IgM, IgA, IgD, IgE, serum and urine-free light chains, or bi- or triclonal gammopathy (see **Table 67–1**).
- IgG occurs in 70% of people, IgM in 15%, and IgA in 10%. A few percent have biclonal or triclonal Ig proteins or solely monoclonal light chains in the plasma and urine (Bence Jones proteinuria).
- In IgG monoclonal gammopathy, the concentration of M protein is usually less than 3.0 g/dL and in IgA and IgM less than 2.5 g/dL, but there are exceptions to this rule.
- Features of a B-cell malignancy are absent.
- Patients with monoclonal gammopathy usually have normal polyclonal immunoglobulin levels as opposed to patients with myeloma or macroglobulinemia, who do not.
- Blood cell counts and marrow examination are normal. The proportion of plasma cells in marrow is usually less than 5%.
- Plasma cell labeling index is low (< 1%).
- Blood T-lymphocyte subsets are normal.
- Serum β_2-microglobulin level is not elevated.
- Marrow microvessel density is three times that of normal individuals but less than that of marrow vasculature in patients with myeloma (although some overlap occurs).
- Interphase fluorescence in situ hybridization frequently uncovers numerical abnormalities (monosomy or trisomy) of chromosomes but progression to a symptomatic B-cell disease is

not correlated with presence or absence of hyperdiploidy or hypodiploidy.

OLIGOCLONAL IMMUNOGLOBULINS

- Oligoclonal immunoglobulins are detected by high-resolution electrophoresis in patients with acute-phase reactants or polyclonal hyperglobulinemia.
- These immunoglobulins are frequent in the CSF of patients with neurologic conditions (eg, multiple sclerosis).
- Serum oligoclonal or monoclonal immunoglobulins occur in patients with the acquired immunodeficiency syndrome (AIDS).

NEUROPATHIES AND MONOCLONAL GAMMOPATHY

Occurrence, Clinical, Laboratory, and Pathologic Findings

- Approximately 4% of patients with monoclonal gammopathy have neuropathy.
- IgM monoclonal gammopathy is more strongly associated with neuropathy than IgG or IgA.
- Approximately 10% of patients with idiopathic neuropathy have a monoclonal protein, a frequency about eight times that of age-matched comparison groups.
- Pathogenesis unclear (see *Williams Hematology*, 9th ed, Chap. 107, for discussion).
- Dysesthesias of hands and feet, loss of vibration and position sense, ataxia, intention tremor, and atrophy of distal muscle groups can occur, especially in association with IgM gammopathy.
- Patients with IgG or IgA gammopathy usually have chronic inflammatory demyelinating neuropathy. A minority have sensory axonal or mixed neuropathy.
- Severity of neuropathy can range from (1) mild with minor motor and/or sensory signs with or without mild functional impairment; (2) moderately disabling but with full range of activities; or (3) severely disabling, interfering with walking, dressing, and eating.
- The course may be remitting or progressive.
- IgA gammopathy may be associated with dysautonomia.
- Decreased nerve conduction velocity indicates demyelinization; decreased sensory potentials indicates axonal loss; and electromyography may indicate denervation of muscles.
- Nerve biopsies may detect demyelinization of nerve fibers or axonal degeneration.

Management

- At least seven approaches have been used to improve the neuropathic findings: (1) intravenous IgG; (2) glucocorticoids; (3) immunoabsorption of perfused blood with staphylococcal protein A; (4) plasma exchange or plasmapheresis; (5) immunosuppressive cytotoxic chemotherapy with cyclophosphamide, fludarabine, or chlorambucil with or without glucocorticoids; (6) rituximab; and (7) high-dose cytotoxic therapy with autologous hematopoietic stem cell rescue.
- Plasma exchange (acute benefit) followed by immunosuppressive chemotherapy (chronic benefit) is a sequence sometimes used.
- In patients with IgM and neuropathy, therapists may start with intravenous IgG as a less toxic initial approach.

- Response rates to each type of therapy are low and the duration of response unpredictable.
- Mild symptoms may not be an indication for therapy because of the low response rate and the noxious effects of therapy.

COINCIDENTAL DISORDERS

- Essential monoclonal gammopathy has been reported coincidental to a large number of conditions (see **Table 67–3**).
- Because the incidence of essential monoclonal gammopathy increases with age, other disorders that increase with age may be expected to coassociate without having any pathogenetic relationship, and few studies have examined formally whether a causal relationship exists.
- Gaucher disease is one disorder in which a pathogenetic relationship exists.
- Obesity has been associated with an increased incidence of essential monoclonal gammopathy (and myeloma).

TABLE 67–3 DISORDERS REPORTED IN COINCIDENCE WITH MONOCLONAL GAMMOPATHY

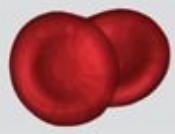
Axial bone fracture
Connective tissue diseases and autoimmune diseases: Crohn disease, cryoglobulinemia, Hashimoto thyroiditis, lupus erythematosus, myasthenia gravis, pernicious anemia, polymyalgia rheumatica, psoriatic arthritis, rheumatoid arthritis, scleroderma, Sjögren disease
Corneal and other ocular diseases: pseudo–Kayser-Fleischer ring, corneal gammopathy
Cutaneous diseases: Schnitzler syndrome, urticaria, hyperkeratotic spicules, pyoderma gangrenosum (neutrophilic dermatoses), psoriasis, scleromyxedema
Diffuse idiopathic skeletal hyperostosis
Endocrine diseases: hyperparathyroidism
Gaucher disease, type I
Hepatic disease: cirrhosis, hepatitis
Hereditary spherocytosis
Infectious diseases: bacterial endocarditis, *Corynebacterium* species, cytomegalovirus, Epstein-Barr virus, human immunodeficiency virus, *Mycobacterium tuberculosis*, purpura fulminans
Metabolic disease: hyperlipidemia
Neutropenia, chronic
Osteoporosis
Pituitary macroadenoma
Pregnancy
Systemic capillary leak syndrome
Carcinomas: colon, lung, prostate, other
Myeloproliferative diseases: acute and chronic myelogenous leukemia, chronic neutrophilic leukemia, polycythemia vera
T-cell lymphomas, Hodgkin lymphoma
After chemotherapy, radiotherapy, or marrow, kidney, or liver transplantation
Miscellaneous diseases
Transient, monoclonal, or oligoclonal gammopathies

Factitious hyperferremia
Factitious increase in C-reactive protein
Vitamin B_{12} deficiency

Source: *Williams Hematology,* 9th ed, Chap. 106, Table 106–3.

TREATMENT, COURSE, AND PROGNOSIS

- Patterns of outcome in patients with essential monoclonal gammopathy:
  — Twenty-five percent of patients progress to develop myeloma, amyloidosis, macroglobulinemia, lymphoma, chronic lymphocytic leukemia over a 25-year period of observation. (Approximately 1% per year progress to some form of B-cell malignancy.)
  — Twenty-five percent of patients have a modest increase in Ig protein levels over time but do not progress to a B-cell malignancy.
  — Fifty percent of patients do not have progression (clonal evolution) during their lifetime.
  — Although studies have shown some variables at diagnosis that may predict for earlier progression in groups of patients (eg, higher marrow plasma cell concentrations, higher monoclonal Ig levels, lower levels of polyclonal Ig, abnormal serum light chain ratio), they are not sufficiently predictive in a single patient. In addition, there is no evidence, as yet, that early treatment for an incipient B lymphocyte neoplasm is worthwhile.
  — Currently, neither gene expression analysis nor cytogenetic findings are sufficient to predict time of progression.
  — Rarely, the monoclonal protein disappears spontaneously.
  — Periodic reevaluation is required to determine the stability of the clinical course after diagnosis and to identify evidence of progression during long periods of observation.
- Therapy is generally not required for essential monoclonal gammopathy unless the monoclonal protein impairs the function of a normal plasma (eg, acquired antithrombin) or tissue constituent (eg, neuropathy) (see **Table 67–2**).



For a more detailed discussion, see Marshall A. Lichtman: Essential Monoclonal Gammopathy, Chap. 106, in *Williams Hematology,* 9th ed.

CHAPTER 68

Myeloma

DEFINITION

- Myeloma is a malignancy of terminally differentiated B cells (plasma cells) that produces a complete and/or partial (light chain) monoclonal immunoglobulin protein.
- Clinical and laboratory manifestations are heterogeneous but typically include:
  - A monoclonal immunoglobulin in plasma and/or monoclonal light chains in plasma and urine. In rare cases, the cells do not secrete a monoclonal protein in the plasma
  - Decreased polyclonal immunoglobulin secretion by residual normal plasma cells, which predispose to infections
  - Myeloma cell proliferation in marrow leading to impaired hematopoiesis
  - Osteolytic bone disease
  - Often hypercalcemia as a result of osteolysis
  - Renal dysfunction as a result of light chain casts or hypercalcemia

EPIDEMIOLOGY

- Myeloma accounts for more than 1% of all malignancies and 10% of hematologic neoplasms.
- Most patients are diagnosed between ages 65 to 74 years of age; only 4% of cases occur before 45 years. The median age of onset is 69 years.
- Men are affected more frequently than women (1.6:1 ratio). Individuals of African descent have twice the prevalence as those of European descent.
- Myeloma is always preceded by a condition known as essential monoclonal gammopathy (MG), which may develop years before the diagnosis of myeloma. Among patients with MG, progression to myeloma is 1% per year.
- Genome-wide association studies identified six single nucleotide polymorphisms (SNPs) associated with risk for MG and myeloma, including 2p23.3, 3p22.1, 3q26.2, 6p21.33, 7p15.3, 17p11.2, and 22q13.1. The identified genes (*DNMT3A*, *ULK4*, *TERC*, *PSORS1C1*, *CDCA7L/DNAH1*, *TNFRSF13B*, and *CBX7*) have not been validated as myeloma-driver genes.

ETIOLOGY AND PATHOGENESIS

- Myeloma cells are derived from postgerminal-center marrow plasmablasts/plasma cells. Stages of evolution from MG to plasma cell leukemia are shown in **Figure 68–1**.
- Myeloma cell immunoglobulin heavy chain (IgH) variable genes present somatic mutations in

the absence of intraclonal variation or ongoing somatic hypermutation.

- Myeloma is characterized by karyotypic abnormalities including translocations and copy change numbers. Common genomic aberrations are shown in **Table 68–1**.
- DNA hyperdiploidy is present in up to 60% of patients.
- Hyperdiploid myeloma patents, typically IgG kappa-type with bone involvement, show gains of odd-numbered chromosomes, including 15, 9, 5, 19, 3, 11, 7, and 21 (ordered by decreasing frequency).
- Nonhyperdiploid myeloma usually is associated with *IgH* gene translocations located at chromosome 14q32 and in some patients with translocations involving the λ light chain locus on chromosome 22. Translocations involving the κ locus on chromosome 2 are rare.
- Deletions of chromosomes 13 (resulting in *RB1* gene and miRNA-15a/16-1 cluster dysregulation) and 17 (involving the *TP53* locus), and amplification of chromosome 1q21 have been associated with a poor prognosis.
- The interaction of myeloma cell with the marrow microenvironment plays a key role in disease progression and drug resistance.

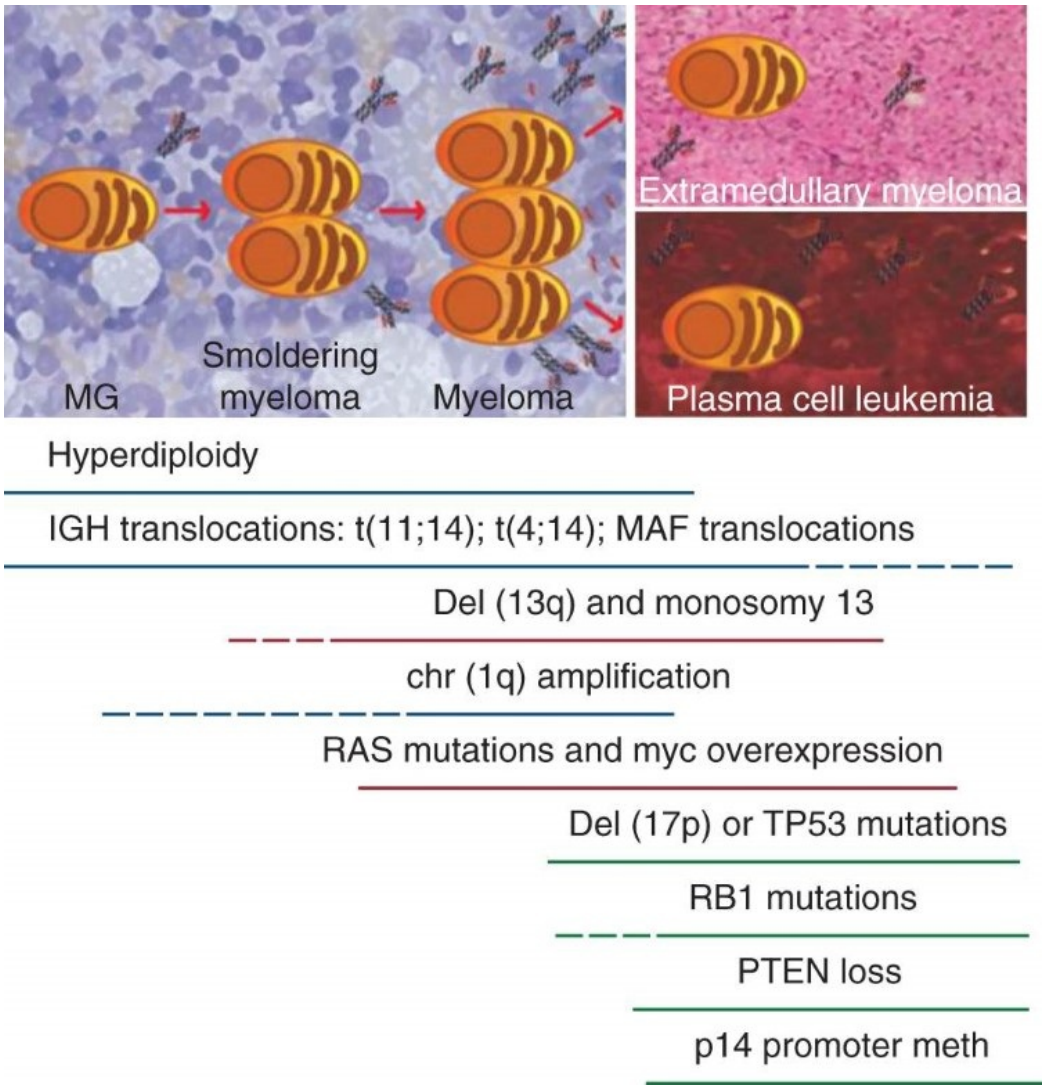


FIGURE 68–1 Myeloma stages, from essential monoclonal gammopathy (MG) to plasma cell leukemia. Myeloma evolves from a benign condition called essential monoclonal gammopathy (or monoclonal gammopathy of undetermined significance), with an annual rate of progression of 1%. In some patients, a stage called smoldering myeloma is sometimes evident, where there is a higher number of monoclonal plasma cells in the marrow but still absence of symptoms. At early stages during the so-called intramedullary phase, myeloma cells are totally dependent on marrow microenvironment to survive and on interleukin-6 and other

cytokines. During progression, myeloma cells can acquire the capability of growing without microenvironmental support and localize to other tissues (extramedullary disease) or circulate in the blood (secondary plasma cell leukemia). Active myeloma is characterized by onset of angiogenesis and bone lytic lesions in contrast to MG or smoldering myeloma; during late stages there is an increase in migration and invasion capabilities, as well as high proliferative rates. (Source: *Williams Hematology*, 9th ed, Chap. 107, Fig. 107–1.)

TABLE 68–1 COMMON GENOMIC ABERRATIONS IN ESSENTIAL MONOCLONAL GAMMOPATHY, MYELOMA, AND PLASMA CELL LEUKEMIA*

Genetic Lesion	MG	Myeloma	Plasma Cell Leukemia
Hyperdiploidy	50%	60%	20%
t(11;14)	5%–10%	20%	25%–60%
t(4;14)	2%–3%	15%	15%–25%
MAF translocations		5%	15%–35%
Del(13q)/Monosomy 13	20%	50%–60%	60%–80%
Del(1p)	4%	7%–40%	
Chr 1q21 amplification		40%	70%
Cyclin D dysregulation	60%	80%	
RAS mutations	< 5%	30%–50%	30%
FAM46C, DIS3		10%–21%	
NF-κB activating mutations and CNVs		15%–20%	
IGH *MYC* rearrangements	1%–2%	15%	30%–50%
UTX deletions and mutations		30%	
TP53 inactivations (mutations + del(17p))	5%	10%–20%	20%–80%
p18 and/or Rb inactivation		< 5%	25%–30%
p14 promoter methylation		< 5%	25%–30%
PTEN loss	0%	< 2%	8%–33%

CNV, copy number variant; IGH, immunoglobin heavy chain; MG, essential monoclonal gammopathy; NF-κB, nuclear factor-kappaB; Rb, retinoblastoma tumor-suppressor protein.

*Myeloma is a multistep process, progressing from an indolent MG stage, to overt myeloma to plasma cell leukemia. Hyperdiploidy and IGH translocations [t(11;14), t(4;14) and MAF translocations] are present at similar rates in MG and myeloma. Conversely, *MYC* secondary rearrangements, deletion 13p, chromosome 1 abnormalities, and *RAS* mutations are more common in active myeloma and they have been postulated as driver myeloma events. Plasma cell leukemia shows distinct abnormalities, including p14 promoter methylation and *PTEN* losses. Frequencies of common genomic aberrancies in plasma cell dyscrasias are reported. Blank spaces are left in case of unknown data.

Source: *Williams Hematology*, 9th ed, Chap. 107, Table 107–1.

CLINICAL AND LABORATORY FEATURES

- Criteria for diagnosis of myeloma are shown in **Table 68–2**.
- The most critical criterion of symptomatic disease and, hence, initiation of therapy, is evidence of organ or tissue impairment (end-organ damage) manifested by anemia, hypercalcemia, lytic bone lesions, renal insufficiency, hyperviscosity, amyloidosis, or recurrent infections, commonly referred to as “CRAB” criteria for hyper*c*alcemia, *r*enal failure, *a*nemia, and *b*one lesions.

TABLE 68–2 CRITERIA FOR DIAGNOSIS OF MYELOMA

1. Clonal bone marrow plasma cells ≥ 10% of biopsy-proven bony or extramedullary plasmacytoma
2. Any one or more of the following myeloma-defining events:
   - Evidence of end-organ damage that can be attributed to the underlying plasma cell proliferative disorder, specifically:
     - Hypercalcemia: serum calcium > 0.25 mmol/L (> 1 mg/dL) higher than the upper limit of normal or > 2.75 mmol/L (> 11 mg/dL)
     - Renal insufficiency: creatinine clearance < 40 mL per min or serum creatinine > 177 μmol/L (> 2 mg/dL)
     - Anemia: hemoglobin value of > 20 g/L below the lower limit of normal, or a hemoglobin value < 100 g/L
     - Bone lesions: one or more osteolytic lesions on skeletal radiography, CT, or PET-CT
   - Any one or more of the following biomarkers of malignancy:
     - Clonal bone marrow plasma cell percentage* ≥ 60%
     - Involved: uninvolved serum free light chain ratio ≥ 100
     - > 1 focal lesions on MRI studies

*Clonality should be established by showing kappa/lambda-light-chain restriction on flow cytometry, immunohistochemistry, or immunofluorescence. Bone marrow plasma cell percentage should preferably be estimated from a core biopsy specimen; in case of a disparity between the aspirate and core biopsy, the highest value should be used.
Source: *Williams Hematology*, 9th ed, Chap. 107, Table 107–2.

Hematologic Abnormalities

- Myelomatous involvement of the marrow typically causes anemia, but when advanced may also contribute to neutropenia and thrombocytopenia.
- Overproduction of interleukin (IL)-6 by marrow stroma, normal accessory cells, and/or myeloma cells may contribute to the anemia by upregulating hepatic production of hepcidin (see Chap. 9), which blocks release of iron from macrophages and inhibits iron absorption from the intestine.
- Thrombocytopenia is uncommon in early phases of myeloma, even with extensive marrow myeloma cell replacement.
- Most patients have an inappropriate erythropoietin response for the level of their anemia.
- Bleeding occurs in 15% of patients with IgG myeloma and in 30% of patients with IgA myeloma.
- Thrombocytosis should alert one to the possibility of hyposplenism because of amyloid deposition in the spleen.
- Hypercoagulable states may result from defective fibrin structure and fibrinolysis because of increased immunoglobulin levels; increased acquired protein C resistance; and increased synthesis of proinflammatory cytokines, such as IL-6.
- Lupus anticoagulants have been reported in association with myeloma.

Immunoglobulin Abnormalities

- Virtually all patients with myeloma secrete a monoclonal immunoglobulin (M-protein or M-spike) that can be detected by immunofixation analysis of the serum and/or urine.
  — Approximately 60% of myeloma patients have detectable monoclonal IgG (usually > 3.5 g/dL), 20% have monoclonal IgA (typically > 2 g/dL), and 20% have only monoclonal immunoglobulin light chains. Excess light-chain proteinuria, however, can accompany IgG, IgA, and, especially, IgD myeloma.

- As a result of the advent of highly sensitive assays to detect serum free light chains (FLCs), an extremely small proportion of patients may have nonsecretory myeloma (no apparent extracellular monoclonal protein).
- Myelomas producing monoclonal IgD, IgE, IgM, or more than one immunoglobulin class are rare.
- The presence of a low concentration of serum monoclonal immunoglobulin by zonal electrophoresis should alert to the possibility of IgD myeloma, especially when associated with excess λ light chains in the serum and light-chain proteinuria, as 80% of IgD myeloma are of the λ light-chain variety.
  — Even patients with light-chain type myeloma, nonsecretory myeloma, or IgD or IgE myeloma often have depressed levels of normal, polyclonal serum IgG, IgA, and IgM.
- The half-life of FLCs is 2 to 4 hours, providing a means to assess the effects of therapy on the myeloma cell mass more quickly than by following serum immunoglobulin.
- Intact immunoglobulins have a half-life of 17 to 21 days, and responses in immunoglobulin levels to therapy are much slower to become apparent.

Marrow Findings

- The marrow can be diffusely infiltrated but commonly displays considerable site-to-site variation in myeloma cell density in a given patient (focal or nodular involvement).
- Cytologically, myeloma cells resemble plasma cells, exhibiting varying degrees of maturity (**Figure 68–2**).
- Myeloma cells produce either κ or λ light chains, which are present in the cytoplasm but not on the membrane surface (**Figure 68–3**).
- Myeloma cells are normally CD138+, CD45-, CD38+, and CD19-, and are CD56+ in 70% of patients.
- Secondary myelodysplasia (dysmorphic red cells, granulocytes, and megakaryocytes) can rarely develop after prolonged treatment in myeloma patients, particularly with alkylating agents (see Chap. 44).
- Metaphase cytogenetic studies and interphase fluorescent in situ hybridization (FISH) analysis should be performed routinely at diagnosis.
- Plasma cell labeling index, as determined by tritiated thymidine or bromodeoxyuridine techniques, that exceeds 0.5% is associated with a relatively short survival.

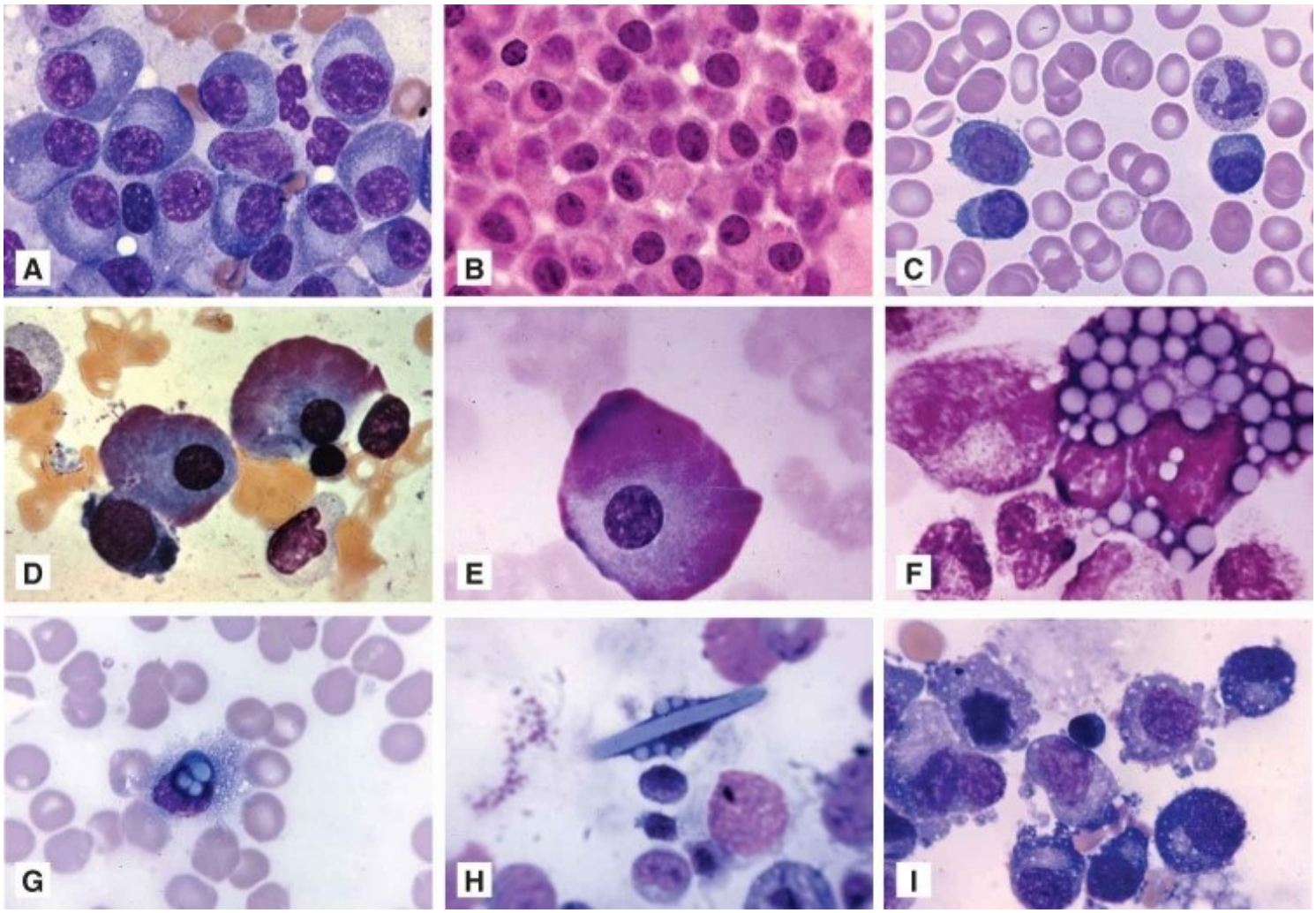


FIGURE 68–2 Myeloma: morphologic appearances. **A.** Marrow film showing replacement by malignant plasma (myeloma) cells. Note classical oval cell shape, eccentric nucleus, striking paranuclear clear area, and deeply blue cytoplasm. **B.** Marrow biopsy section showing replacement by myeloma cells. **C.** Blood film in a patient with plasma cell leukemia. Three myeloma cells in the blood film field. **D** and **E.** Marrow films. Flaming-type giant myeloma cells. Reddish peripheral cytoplasmic coloration reflecting very high concentration of carbohydrate, characteristic of IgA myeloma. The peripheral cytoplasm contains numerous dilated cisterns of the endoplasmic reticulum distended with immunoglobulin. Flaming plasma cells may occasionally be found in IgG myeloma and in reactive plasmacytosis. **F.** Morula or Mott cell. Myeloma cell engorged with globules, presumably containing immunoglobulin. These globules individually are referred to as Russell bodies, and plasma cells may be found containing one, several, or many such bodies. **G.** Plasma cell with immunoglobulins containing globules overlying the nucleus but presumably cytoplasmic in location along with smaller cytoplasmic globular inclusions. **H.** Immunoglobulin crystal with several globules of immunoglobulin on either side. Note remarkable distortion of the cell to accommodate the crystal. **I.** Marrow film. Myeloma cells exhibiting cytoplasmic shedding. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

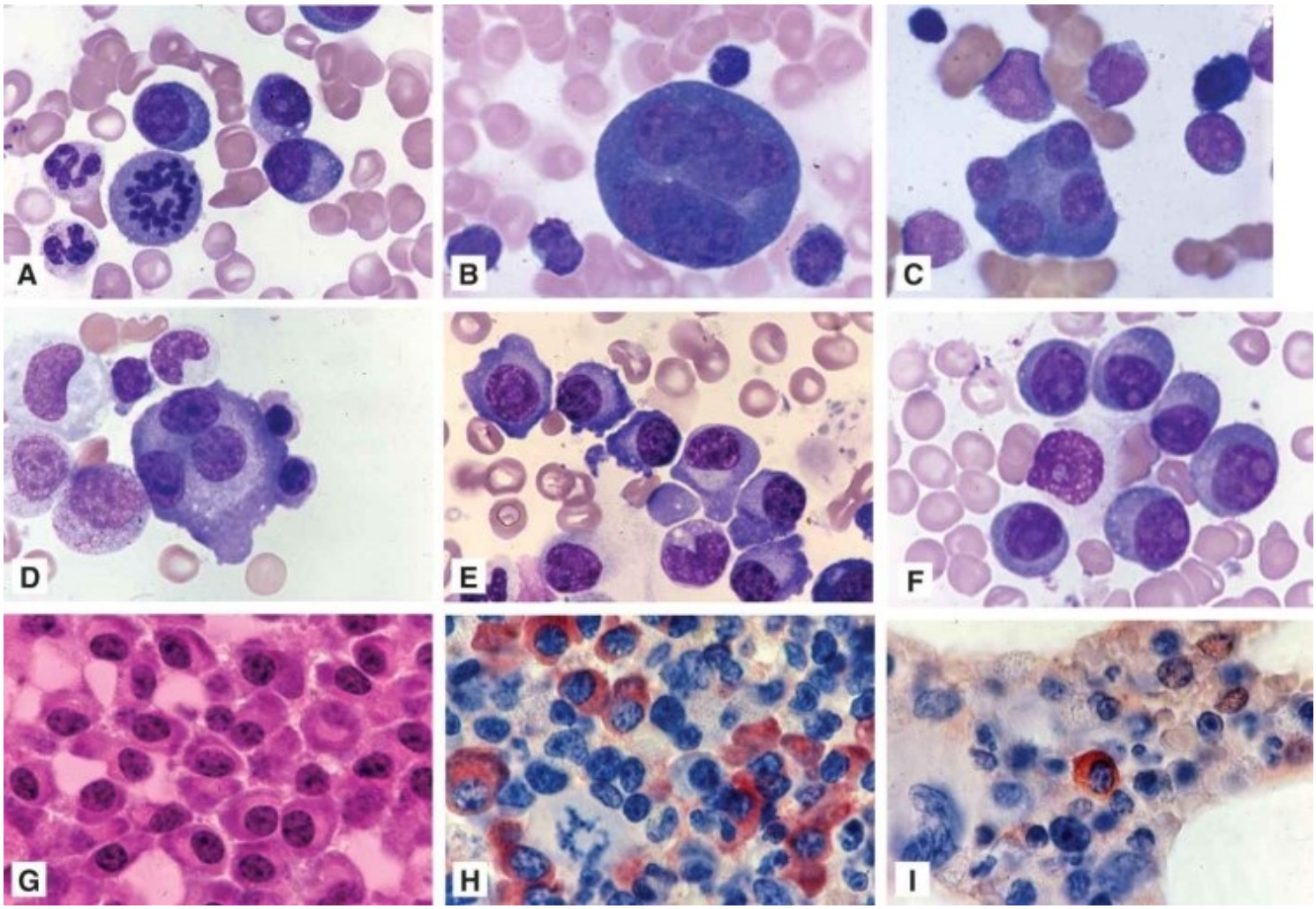


FIGURE 68–3 Myeloma: Additional morphologic appearances. **A.** Marrow film. Three characteristic malignant plasma (myeloma) cells and one in mitosis. **B.** Marrow film. Giant multinucleated myeloma cell. **C.** Marrow film. Tetranucleated myeloma cell. **D.** Marrow film. Trinucleated myeloma cell. **E.** Marrow film. Infiltrate of classical well-differentiated myeloma cells (plasma cell phenocopies). Eccentric nuclei, paranuclear clear zone, and deeply blue (basophilic) peripheral cytoplasm. **F.** Infiltrate of immature myeloma cells with more circular than ovoid shapes, very large, prominent large nucleoli, less-intense basophilic cytoplasm, and less-discrete paranuclear clear zone (plasmablasts). **G** to **I.** Marrow biopsy sections showing striking infiltrate of myeloma cells. **G.** Hematoxylin-and-eosin stain. **H.** Immunostained for κ light chains, showing frequently positive cells evident by deep rust color in cytoplasm. **I.** Immunostained for λ light chains showing negative reaction with rare positive cell. Approximately 20:1 κ:λ ratio. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

Renal Disease

- Some form of renal impairment occurs in 30% to 50% of myeloma patients at diagnosis, with up to 10% of patients requiring hemodialysis.
- Myeloma cast nephropathy is the most common cause of renal impairment and is also referred to as myeloma kidney.
  - — Myeloma kidney is most commonly caused by the formation of tubular casts in the distal nephron formed by the binding of light chains to uromodulin (Tamm-Horsfall protein).
  - — There is considerable variation in the nephrotoxic proclivity of light chains (eg, λ light chains are more nephrotoxic than the κ type).
- The second most common cause of renal impairment is hypercalcemia, which is present in 15% of patients at diagnosis. Hypercalcemia creates volume depletion, natriuresis, and renal vasoconstriction.
- AL amyloidosis associated with light-chain immunoglobulin proteinuria usually presents as the

nephrotic syndrome, with very little light-chain secretion in the urine, but can lead, over time, to renal failure (see Chap. 71).
  — Amyloid deposits can be detected by Congo red staining.
  — AL amyloidosis is more common in patients with λ light-chain myeloma proteins than in patients with κ light-chain myeloma, especially those with λ light-chain proteins that have immunoglobulin variable regions belonging to the λ VI light-chain subgroup.
- The differential diagnosis of nephrotic syndrome in the myeloma patient should include renal vein thrombosis.
- Therapy of myeloma renal impairment, in addition to supportive care, includes (1) correction of hypercalcemia with aggressive hydration and calcitonin and (2) slow infusion of a single dose of a bisphosphonate. Cytoreductive therapy should be started as soon as possible. High cutoff hemodialysis using filters that remove light chains with great efficiency have resulted in improved patient outcomes.
- In general, myeloma renal impairment is reversible in approximately 50% of patients.

Pain

- Back or chest bone pain from factures or lytic bone lesions is present in 60% of patients at diagnosis.
- Localized pain can derive from focal plasmacytomas or amyloid deposits into nerve sheaths.

Infections

- Infection is a leading cause of morbidity and mortality in myeloma patients.
- Hypogammaglobulinemia, reflecting suppression of CD19+ B lymphocytes, results in susceptibility to encapsulated organisms such as *Streptococcus pneumoniae* and *Haemophilus influenzae*.
- Deficiencies in cellular immune function account for the recurrent infections commonly seen.
- Abnormalities in T-cell function include reversed CD4+/CD8+ T-cell ratios, severe disruptions in the T-cell repertoire, and abnormal intracellular signal transduction impairing T-cell activation.
- In patients with persistently low CD4+ counts, *Pneumocystis jiroveci* prophylaxis should be considered.
- Yearly influenza vaccination and a single pneumococcal vaccination is recommended at diagnosis.

Neuropathy

- Neurologic abnormalities generally are caused by regional myeloma cell growth compressing the spinal cord or cranial nerves.
- Polyneuropathies are observed with perineuronal or perivascular (*vasa nervorum*) amyloid deposition.

Hyperviscosity

- Hyperviscosity occurs in less than 10% of patients with myeloma.
  — Symptoms of hyperviscosity result from circulatory problems, leading to cerebral,

pulmonary, renal, and other organ dysfunction.
— Patients with IgA myeloma have hyperviscosity more frequently than do patients with IgG myeloma.
— Among patients with IgG myeloma, those with tumors expressing immunoglobulins of the IgG_3 subclass are the most susceptible.

Extramedullary Disease

- Plasma cell leukemia is diagnosed when more than 2.0×10^9/L myeloma cells are present in the blood or plasmacytosis accounts for more than 20% of the differential white cell count. It is rare at presentation but can develop in approximately 5% of patients as a terminal disease manifestation.
- Visceral organ involvement of liver, lymph nodes, spleen, kidneys, breasts, pleura, meninges, and cutaneous sites should be suspected in the presence of elevated serum levels of lactic acid dehydrogenase (LDH).

Spinal Cord Compression

- Spinal cord compression can result from an extramedullary plasmacytoma or vertebral fracture. It should be considered a medical emergency, evaluated with magnetic resonance imaging (MRI), and promptly treated.
- In the presence of a solitary plasmacytoma, local radiotherapy using less than 30 Gy can be curative.
- In patients with systemic disease, chemotherapy that includes high-dose dexamethasone pulsing, as part of the combination oral dexamethasone, daily thalidomide, and 4 days of continuous-infusion cisplatin, doxorubicin, cyclophosphamide, and etoposide (DT PACE) is effective.
  — In the absence of symptom relief and lack of tumor shrinkage on MRI within 1 week, local radiation should be added.
  — If cord compression results from vertebral collapse without identifiable plasmacytoma on MRI, radiation may not be beneficial, and decompressive laminectomy may be the treatment of choice.

INITIAL EVALUATION OF THE PATIENT WITH MYELOMA

- Minimal evaluation requirements include evaluation of the complete blood count with white cell differential count, myeloma protein studies, examination of the blood film for the presence of pathological rouleaux or circulating myeloma cells, a comprehensive serum metabolic panel for the detection of hypercalcemia and azotemia, serum β_2-microglobulin, C-reactive protein, and LDH (**Table 68–3**).
- Myeloma protein studies should include:s
  — Serum protein electrophoresis
  — Serum free light-chain assay, as well as a 24-hour urine collection to quantify 24-hour total urinary protein and measure quantity of light chains

— Immunofixation of serum and urine, which is needed for the immunoglobulin heavy- and light-chain isotype determination

- Marrow aspiration and biopsy should include genetic studies (eg, FISH and cytogenetics) and flow cytometry.
- Bone imaging should include a complete skeletal survey. MRI and fluoro-2-deoxyglucose (FDG) positron emission tomography–computed tomography (PET-CT) are more sensitive and better capture early bone disease, the extent of bone disease, and extramedullary disease.
- Assessment of the heart in the proper clinical context by echocardiogram and electrocardiogram is useful to detect cardiac amyloidosis.
- Measurement of brain natriuretic peptide and N-terminal prohormone B-type natriuretic peptide are useful screening tests to detect cardiac dysfunction caused by amyloidosis or light chain deposition disease, or LCDD.

TABLE 68–3 ASSESSMENT OF MYELOMA

Complete blood count and differential count; examination of blood film
Chemistry screen, including calcium, creatinine, lactate dehydrogenase, BNP, proBNP
β2-Microglobulin, C-reactive protein
Serum protein electrophoresis, immunofixation, quantification of immunoglobulins, serum-free light chains
24-Hour urine collection for protein electrophoresis, immunofixation, quantification of immunoglobulins, including light chains
Marrow aspirate and trephine biopsy with metaphase cytogenetics, FISH, immunophenotyping; gene array, and plasma cell labeling index (if available)
Bone survey and MRI; PET-CT (if available)
Echocardiogram with assessment of diastolic function and measurement of interventricular septal thickness; EKG (if amyloidosis suspected)

BNP, brain natriuretic peptide; CT, computed tomography; EKG, electrocardiogram; FISH, fluorescence in situ hybridization; MRI, magnetic resonance imaging; PET, positron emission tomography; proBNP, prohormone B-type natriuretic peptide.
Source: *Williams Hematology*, 9th ed, Chap. 107, Table 107–4.

Staging

- The Salmon-Durie staging system has been in use for more than 30 years but is being replaced by newer staging systems (see **Table 68–4**).
- The international staging system (ISS) is based on two widely available parameters, serum β_2-microglobulin and albumin, and recognizes three stages (see **Table 68–5**). Shortcomings of the ISS include lack of accounting for cytogenetics and bone disease.

TABLE 68–4 ASSSESSMENT OF MYELOMA TUMOR MASS (SALMON-DURIE)

I. High tumor mass (stage III) (> 1.2×10^{12} myeloma cells/m^2)*
One of the following abnormalities must be present:
A. Hemoglobin < 8.5 g/dL, hematocrit < 25%
B. Serum calcium > 12 mg/dL
C. Very high serum or urine myeloma protein production rates:
1. IgG peak > 7 g/dL
2. IgA peak > 5 g/dL
3. Urine light chains > 12 g/24 h
D. More than three lytic bone lesions on bone survey (bone scan not acceptable)

II. Low tumor mass (stage I) (< 0.6×10^{12} myeloma cells/m^2)*
All of the following must be present:
A. Hemoglobin > 10.5 g/dL or hematocrit > 32%
B. Serum calcium normal
C. Low serum myeloma protein production rates:
1. IgG peak < 5 g/dL
2. IgA peak < 3 g/dL
3. Urine light chains < 4 g/24 h
D. No bone lesions or osteoporosis

III. Intermediate tumor mass (stage II) (0.6 to 1.2×10^{12} myeloma cells/m^2)*
All patients who do not qualify for high or low tumor mass categories are considered to have intermediate tumor mass
A. No renal failure (creatinine ≤ 2 mg/dL)
B. Renal failure (creatinine > 2 mg/dL)

*Estimated number of neoplastic plasma cells.
Reproduced with permission from Durie BG, Salmon SE. A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment, and survival, *Cancer* 1975 Sep;36(3):842-54.

TABLE 68–5 INTERNATIONAL STAGING SYSTEM

Stage I	β_2M < 3.5 ALB ≥ 3.5
Stage II	β_2M < 3.5 ALB < 3.5 or β_2M 3.5–5.5
Stage III	β_2M > 5.5

ALB, serum albumin in g/dL; β2M, serum β2-microglobulin in mg/L.
Data from Greipp PR, San Miguel J, Durie BG, et al. International staging system for multiple myeloma, *J Clin Oncol* 2005 May 20;23(15):3412-3420.

Imaging Studies

- Standard of care in initial staging is a complete skeletal survey that includes posteroanterior view of the chest; anterior-posterior and lateral views of the skull, spine, and pelvis; rib series; and long bone images.
- Roentgenographically detectable osteolytic lesions require at least 50% to 70% loss of bone mass and represent advanced bone destruction; thus conventional x-rays have limited sensitivity.
- MRI detects focal intramedullary disease in two-thirds of patients at time of diagnosis, even before the onset of bone destruction. MRI is more sensitive than the skeletal survey and is now widely used in both newly diagnosed and relapsed myeloma, as well as in event of suspected cord compression.
- PET-CT scans are able to detect lesions at least 1 cm in diameter; active disease may be identified before bony destruction.

TREATMENT

Management of Newly Diagnosed Myeloma

- Every newly diagnosed myeloma patient should be assessed for fitness to undergo an autologous hematopoietic stem cell transplant. Performance status, organ function, and comorbidities, rather than age alone, should be considered.
- Autologous transplantation achieves a complete response rate of 40%, but a median duration of response of only 2 to 3 years. Whether tandem autologous transplantation can improve on these results is being addressed in a Blood and Marrow Transplant Clinical Trials Network (BMT CTN) phase III, multicenter trial.
- **Table 68–6** lists novel agents and combinations used for induction in newly diagnosed transplant-eligible patients. Regimens that include alkylating agents should be avoided, because these drugs damage normal hematopoietic stem cells and make stem cell harvesting more difficult.
- **Table 68–7** lists induction regimens for newly diagnosed transplant-ineligible patients.

TABLE 68–6 NOVEL AGENT INDUCTION FOR NEWLY DIAGNOSED TRANSPLANT-ELIGIBLE PATIENTS

Study	Regimen	No. of Patients	CR/nCR (%)	ORR (%)	Outcome
Rajkumar et al	RD	223	18	79	OS 96% on Rd vs 87% on RD at 1-year
	Rd	222	14	68	
Harousseau et al	VAD	121	6.4	62.8	PFS 36 mo Bd vs 30 mo VAD at 32 mo
	Bd	121	14.8	78.5	
Reeder et al	CyBorD	33	39	88	N/A
Richardson et al	RVD	66	39	100	OS 97% at 18 mo
Jakubowiak et al	CRD	53	62	98	PFS 92% at 24 mo

Bd, bortezomib, low-dose dexamethasone; CR, complete resonse; nCR, near complete response; CRD, carfilzomib, lenalidomide, dexamethasone; CyBorD, cyclophosphamide, bortezomib, dexamethasone; N/A, not available; ORR, overall response rate; OS, overall survival; PFS, progression free survival; RD, lenalidomide, high-dose dexamethasone; Rd, lenalidomide, low-dose dexamethasone; RVD, lenalidomide, Velcade, dexamethasone; VAD, vincristine, Adriamycin, dexamethasone.
Source: *Williams Hematology*, 9th ed, Chap. 107, Table 107–7.

TABLE 68–7 NOVEL AGENT INDUCTION FOR NEWLY DIAGNOSED TRANSPLANT-INELIGIBLE PATIENTS

Study	Regimen	No. of Patients	Median Follow-up (months)	Median OS (months)	Median PFS (months)
IFM 99–06	MP	196	51.5	33.2	17.8
	MPT	125		51.6	27.5
	MEL100	126		38.3	19.4
IFM 01/01	MPT	113	47.5	44	24.1
	MP	116		29.1	18.5
MM-015	MPR-R	152	30	45.2	31
	MPR	153		NR	14
	MP	154		NR	13
VISTA	VMP	344	60	56.4	N/A

	MP	338		43.1	N/A
FIRST	Rd	536	37	59.4	25.5
	Rd18	541		55.7	20.7
	MPT	547		51.4	21.2

MEL 100, melphalan 100 mg/m2; MP, melphalan and prednisone; MPR, melphalan, prednisone, and lenalidomide; MPR-R, melphalan, prednisone, and lenalidomide induction followed by lenalidomide maintenance; MPT, melphalan, prednisone, and thalidomide; NR, not reached; OS, overall survival; PFS, progression-free survival; Rd, lenalidomide and low-dose dexamethasone continuously; Rd18, lenalidomide and low-dose dexamethasone for 18 cycles; VMP, bortezomib, melphalan, and prednisone.
Source: *Williams Hematology*, 9th ed, Chap. 107, Table 107–8.

Maintenance Therapy

- Maintenance regimens, particularly with lenalidomide, have been proposed to extend the duration of complete remission following autologous transplantation.
- A significant concern with maintenance therapy with lenalidomide is the risk of secondary malignancy, which has been reported to nearly double the risk of second primary cancers, including hematologic malignancies and solid tumors.
- **Table 68–8** summarizes maintenance trials.

TABLE 68–8 MAINTENANCE THERAPIES

Study	Regimen	No. of Patients	Outcome
IFM 2005–02	Lenalidomide vs placebo as maintenance following first or second ASCT	614	PFS 41 vs 23 months
CALGB 100104	Lenalidomide vs placebo as maintenance therapy after ASCT	460	TTP 46 vs 27 months
HOVON-65/GMMG-HD4	VAD vs PAD followed by ASCT, then thalidomide or bortezomib as maintenance	827	PFS 28 vs 35 months

ASCT, autologous stem cell transplantation; PAD, bortezomib, doxorubicin, and dexamethasone; PFS, progression-free survival; TTP, time to progression; VAD, vincristine, doxorubicin, and dexamethasone.
Source: *Williams Hematology*, 9th ed, Chap. 107, Table 107–9.

Approach to Patients with Relapsed or Refractory Disease

- **Table 68–9** summarizes trials of novel therapies for relapsed or refractory disease.

TABLE 68–9 NOVEL THERAPIES FOR RELAPSED OR REFRACTORY MYELOMA

Trial	Phase	Agent	No. of Patients	ORR (%)	OS (months)	Outcome (months)
Richardson et al	III	Bortezomib	669	43	29.8	TTP 6.2 vs 3.5
		Dexamethasone		18	23.7	
Orlowski et al	III	Bort/PLD	646	44	76%*	TTP 9.3 vs 6.5
		Bortezomib		41	65%*	
Weber et al	III	Lenalidomide	353	61	29.6	TTP 11.1 vs 4.7
		Dexamethasone		20	20.2	
Dimopoulos et al	III	Lenalidomide	351	60	NR	TTP 11.3 vs 4.7
		Dexamethasone		24	20.6	

Richardson et al	II	RVD	64	64	26	Median TTP 9.5
Siegel et al	II	Carfilzomib	266	24	15.6	Median PFS 3.7
San Miguel et al	III	Pom/LoDex	302	31	12.7	Median PFS 4.0 vs 1.9
		Pom/HiDex		10	8.1	
Dimopoulos et al	III	Vor/Bort	637	56	NR	Median PFS 7.6 vs 6.8
		Bort		41	28.1	
Richardson et al	III	Pan/Bort/Dex	768	61	NR	Duration of response 12 vs 8.1
		Bort/Dex		55		
Lokhorst et al	I/II	Daratumumab	32	42†	NR	Median PFS NR
Lonial et al	II	Elo/Len/Dex	73	92‡	NR	Median PFS NR‡
Lentzsch et al	II	Benda/Len/Dex	29	52	NR	Median PFS 6.1

Benda, bendamustine; Bort, bortezomib; Dex, dexamethasone; Elo, elotuzumab; Hi, high dose; Lo, low dose; NR, not reported/reached; Pan, panobinostat; PLD, pegylated liposomal doxorubicin; Pom, pomalidomide; RVD, lenalidomide, bortezomib, and dexamethasone; Vor, vorinostat.

*At 15 months.

†Of those receiving a dose of ≥ 4 mg/kg.

‡Of those receiving dose of 10 mg/kg at 20.8 months.

Source: *Williams Hematology*, 9th ed, Chap. 107, Table 107–10.

COURSE AND PROGNOSIS

Monitoring Disease Markers for Documentation of Response and Relapse

- The criteria for evaluating response, the International Uniform Response Criteria, proposed by the International Myeloma Working Group (IMWG), are shown in **Table 68–10**.
- Survival end points include progression-free survival, event-free survival, and disease-free survival.
- Sequencing-based platforms, quantitative polymerase chain reaction, and multiparametric flow cytometry are being used to detect minimal residual disease and may be of prognostic value.
- Disease features can change over the course of the disease. Clonal evolution occurs, resulting in loss of previously secreted complete immunoglobulin and switch to only light-chain secretion ("Bence Jones escape"). or entire loss of immunoglobulin-secretory capacity, often associated with extramedullary spread, best signified by increased LDH levels and lesions found on PET-CT examination.
  - — Occasionally, unexplained anemia or pancytopenia accompanies disappearing myeloma protein markers, necessitating prompt marrow examination to detect fulminant relapse.
- Monthly determinations of myeloma protein should be performed during induction therapy.
- After two to four induction cycles and prior to high-dose melphalan-based autologous transplantation, the disease should be restaged, including marrow examination with cytogenetics and MRI and/or PET-CT of indicator lesions.
- Disease monitoring should be performed at least every month for the first year and at a minimum of every other month thereafter.

TABLE 68–10 UNIFORM RESPONSE CRITERIA FROM THE INTERNATIONAL MYELOMA

	WORKING GROUP
Response Subcategory*	**Response Criteria**
CR	Negative immunofixation of the serum and urine and disappearance of any soft-tissue plasmacytomas and < 5% plasma cells in marrow.†
sCR	CR as defined above, plus Normal FLC ratio and Absence of clonal cells in marrow† by immunohistochemistry or immunofluorescence.‡
VGPR	Serum and urine M-protein detectable by immunofixation but not on electrophoresis or 90 or greater reduction in serum M-protein plus urine M-protein < 100 mg per 24 h.
PR	> 50% reduction of serum M-protein and reduction in 24-h urinary M-protein by > 90% or to < 200 mg per 24 h. If the serum and urine M-protein are unmeasurable, > 50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria. If serum and urine M-protein are unmeasurable, and serum-free light assay is also unmeasurable, > 50% reduction in plasma cells is required in place of M-protein, provided baseline marrow plasma cell percentage was > 30%. In addition to the above listed criteria, if present at baseline, a > 50% reduction in the size of soft-tissue plasmacytomas is also required.
SD	Not meeting criteria for CR, VGPR, PR, or progressive disease.

CR, complete response; FLC, free light chain; M-protein, monoclonal protein; PR, partial response; sCR, stringent complete response; VGPR, very good partial response.

*All response categories require two consecutive assessments made at any time before the institution of any new therapy; CR, PR, and stable disease categories also require no known evidence of progressive or new bone lesions if radiographic studies were performed. Radiographic studies are not required to satisfy these response requirements.

†Confirmation with repeat marrow biopsy not needed.

‡Presence/absence of clonal cells is based upon the κ:λ ratio of > 4:1 or < 1:2. An abnormal κ:λ ratio by immunohistochemistry and/or immunofluorescence requires a minimum of 100 plasma cells for analysis.

Note: SD is not recommended for use as an indicator of response; stability of disease is best described by providing the time-to-progression estimates.

Source: *Williams Hematology*, 9th ed, Chap. 107, Table 107–11.

Prognosis

- The prognosis of myeloma is determined by three factors:
  - — Patient factors (eg, age, comorbid conditions)
  - — Tumor biology and disease burden (eg, intrinsic cell drug sensitivity)
  - — Type of therapy applied (eg, newer thalidomide derivatives and proteasome inhibitors)
- Cytogenetic findings associated with poor outcome include:
  - — Hypodiploidy and deletions 13q and 17p13
  - — Mutations at locus of the tumor-suppressor gene *TP53*
  - — Gains and translocations of chromosome 1, which are associated with more aggressive and more advanced myeloma
  - — Gain of the 1q21 region (amp1q21), which increases from approximately 40% at diagnosis to 70% at relapse
  - — Both the proportion of cells with 1q21 and the copy number increases at relapse, suggesting the existence of a gene-dosage effect involved in drug resistance.

— The gene *CKS1B* located at 1q21 controls the G1 to S transition of the cell cycle and has been linked to shorter progression-free survival after autologous transplantation.

- Hyperdiploidy, which accounts for almost half of the patients with abnormal cytogenetics and involves nonrandom gains of chromosomes 3, 5, 7, 9, 11, 15, 19, and 21, is associated with chemosensitive disease and better overall survival.
- Translocation t(11;14) also confers a better outcome.
- Interphase FISH does not depend on cycling cells and can detect abnormal cells in as many as 80% to 90% of cases.
  — FISH can also detect cytogenetically silent translocations.
- Gene-expression profiling has the potential to define disease pathogenesis as well as novel prognostic factors and potential therapeutic targets; profiling will have an important impact on the interpretation of future clinical trials.

SPECIAL DISEASE MANIFESTATIONS

IgM Myeloma

- IgM myeloma is distinct from Waldenström macroglobulinemia (see Chap. 69).
- Plasma cells, rather than the lymphoplasmacytic infiltrate, dominate the marrow of myeloma.
- DNA-aneuploidy and the presence of lytic bone lesions support a diagnosis of myeloma.

Solitary Plasmacytoma

- Solitary plasmacytoma of bone (solitary osseous plasmacytoma [SOP]) or soft tissue (extramedullary plasmacytoma [EMP]) requires the absence of indicators of systemic disease, such as marrow plasmacytosis, anemia, or other lytic or soft-tissue lesions.
- Median age of diagnosis of SOP or EMP is approximately 50 years, nearly 10 years less than for myeloma.
- The majority of patients with SOP progress to myeloma; only 50% of patients with EMP do so.
- Local therapy, primarily radiotherapy, with surgery as needed for structural anatomic support is standard treatment of SOP and EMP.
- Rising monoclonal Ig levels in a patient with a history of either SOP or EMP should trigger a workup for either recurrent plasmacytoma or myeloma.

AL Amyloidosis

- When clinical features of congestive heart failure, nephrotic syndrome, malabsorption, coagulopathy, skin rash (oral mucosal rash, “raccoon’s eyes”) or neuropathy are present, a careful search for primary amyloidosis should be carried out (see Chap. 71).

Smoldering Myeloma

- Smoldering myeloma is a form of myeloma in which there is no evidence of end-organ damage at diagnosis.
- There is no role for treating these patients; however, the IMWG has advised that patients with greater than 60% marrow plasma cells, a κ:λ free light chain ratio greater than 100, and two lesions on MRI or PET-CT imaging may benefit from therapy.

EMERGENT COMPLICATIONS OF NEW MYELOMA THERAPY

Venous Thromboembolism

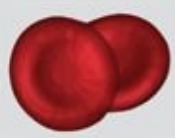
- Patients with myeloma are at an increased risk for deep venous thrombosis and pulmonary embolism, particularly when known risk factors are present. These risk factors are:
  — History of prior venous thromboembolism (VTE), immobilization, and/or dehydration
- Genetic predispositions include high levels of homocysteine; deficiencies of antithrombin, protein C, and protein S; and factor V Leiden or prothrombin gene mutations.
- The incidence of VTE is highest during the first 3 to 4 months following diagnosis and occurs in approximately 3% to 4% of patients receiving either dexamethasone alone or melphalan and prednisone (MP) but is much higher when newer agents are combined with dexamethasone and melphalan.
  — A number of procoagulant abnormalities have been described in myeloma, including endothelial damage, paraprotein interference with fibrin structure, elevated von Willebrand multimers, elevated factor VIII, decreased protein S, and acquired activated protein C resistance.
- The incidence of VTE with single-agent thalidomide is approximately 2% to 4% in newly diagnosed and in relapsed patients, comparable to that observed with dexamethasone alone or MP, implying that thalidomide alone does not increase the risk of VTE.
  — However, the risk of VTE increases significantly when thalidomide is combined with either dexamethasone, melphalan, doxorubicin, or cyclophosphamide, or with other multiagent chemotherapy.
  — Most VTEs occur within the first 60 days of therapy.
- Single-agent lenalidomide does not appear to increase VTE, at least not in the setting of myeloma relapse, but it is associated with a marked increased in VTE risk when lenalidomide is combined with dexamethasone.
- Bortezomib did not seem to increase the risk of VTE.
- Prevention of VTE is based on the assessment for known risk factors for VTE:
  — Myeloma-related (hyperviscosity, newly diagnosed status)
  — Therapy-related (high-dose dexamethasone [≥ 480 mg/month], doxorubicin, multiagent chemotherapy)
  — Individual factors (age, history of VTE, inherited thrombophilia, obesity, immobilization, central venous line, infections, surgery, administration of erythropoietin)
  — Factors related to comorbidities (acute infection, diabetes mellitus, cardiac or renal dysfunction)
- Therapy-related risks factor most in the risk-equation of VTE.
- The following thromboprophylaxis is recommended:
  — Acetylsalicylic acid (aspirin) in either a standard oral dose of 325 mg/day or in a low-dose of 81 mg/day for patients with one or no risk factor
  — Low-molecular-weight heparin, or LMWH, once a day, or full-dose warfarin for patients if two or more risk factors or therapy-related risks are present
- The recommended duration of prophylaxis in general is 6 to 12 months.
- Long-term prophylaxis may be indicated in some patients.

Peripheral Neuropathy

- Bortezomib and thalidomide-induced peripheral neuropathy should be distinguished from other causes, such as paraneoplastic neuropathies, antecedent chemotherapy with neurotoxic agents (vincristine or cisplatinum), diabetes mellitus, or AL amyloidosis.
- If significant weakness or asymmetry of signs is present, a neurologic consultation must be obtained along with electromyography and nerve conduction studies.
- Subcutaneous administration of bortezomib is now the preferred route of administration due to a decreased incidence of peripheral neuropathy compared with intravenous administration.
- Second-generation, more selective proteasome inhibitors such as carfilzomib have reduced neurotoxicity.
- Symptomatic treatment for thalidomide- and bortezomib-induced neuropathy includes gabapentin, pregabalin, or tricyclic antidepressants.

Osteonecrosis of the Jaw

- Osteonecrosis of the jaw is characterized by the presence of exposed bone in the maxillofacial region that does not heal within 8 weeks.
- The exact cause is not known and is likely multifactorial. Bisphosphonates and invasive dental procedures are predisposing factors.
- The risk increases with duration of bisphosphonate exposure (5%–15% at 4 years). Risk is associated with a polymorphism in the cytochrome P450-2C polypeptide; the mechanism is unknown.
- Patents should be referred for dental evaluation prior to beginning bisphosphonates.



For a more detailed discussion, see Elizabeth O'Donnell, Francesca Cottini, Noopur Raje, and Kenneth Anderson: Myeloma, Chap. 107, in *Williams Hematology*, 9th ed.

CHAPTER 69

Macroglobulinemia

DEFINITION

- Waldenström macroglobulinemia (WM) is a lymphoid neoplasm resulting from the accumulation, predominantly in the marrow, of a clonal population of lymphocytes, lymphoplasmacytic cells, and plasma cells, which secrete a monoclonal immunoglobulin (Ig) M.
- WM corresponds to lymphoplasmacytic lymphoma (LPL) as defined in the Revised European–American Lymphoma (REAL) and World Health Organization (WHO) classification systems.
- Most cases of LPL are WM. Less than 5% of cases are IgA-secreting, IgG-secreting, or nonsecreting LPL.

EPIDEMIOLOGY

- The age-adjusted incidence rate of WM in the United States is 3.4 per 1 million among males and 1.7 per 1 million among females.
- The incidence rate is higher among Americans of European descent. Americans of African descent represent approximately 5% of all patients.
- Approximately 20% of patients are of Eastern European descent, specifically of Ashkenazi-Jewish ethnic background.
- Approximately 20% of 257 sequential patients with WM presenting to a tertiary referral center had a first-degree relative with either WM or another B-cell disorder.

PATHOGENESIS

Cytogenetic Findings

- Loss of all or part of chromosomes 17, 18, 19, 20, 21, 22, X, and Y is commonly observed, and gains in chromosomes 3, 4, and 12 also occur.
- Chromosome 6q deletions encompassing 6q21–25 have been observed in up to half of WM patients.

Somatic Mutations

- $MYD88^{L265P}$ and $CXCR4^{WHIM}$ mutations are highly prevalent and trigger transcriptional factors that support the growth and survival of lymphoplasmacytic cells.

CLINICAL FEATURES

- Presenting symptoms most commonly are fatigue, weakness, weight loss, episodic bleeding, and manifestations of the hyperviscosity syndrome.
- Physical findings include:
  — Lymphadenopathy
  — Hepatosplenomegaly
  — Dependent purpura and mucosal bleeding
  — Dilated tortuous retinal veins
  — Multiple flesh-colored papules on extensor surfaces (deposits of IgM reacting to epidermal basement membrane antigens)
  — Peripheral sensory neuropathy
  — Raynaud phenomenon, especially on exposure to cold
  — Splenomegaly and lymphadenopathy (uncommon)

Morbidity Mediated by the Effects of IgM

- **Table 69–1** lists the physiochemical and immunologic properties of the monoclonal IgM protein.

TABLE 69–1 PHYSICOCHEMICAL AND IMMUNOLOGIC PROPERTIES OF THE MONOCLONAL IGM PROTEIN IN WALDENSTRÖM MACROGLOBULINEMIA

Properties of IgM Monoclonal Protein	Diagnostic Condition	Clinical Manifestations
Pentameric structure	Hyperviscosity	Headaches, blurred vision, epistaxis, retinal hemorrhages, leg cramps, impaired mentation, intracranial hemorrhage
Precipitation on cooling	Cryoglobulinemia (type I)	Raynaud phenomenon, acrocyanosis, ulcers, purpura, cold urticaria
Autoantibody activity to myelin-associated glycoprotein, ganglioside M_1, sulfatide moieties on peripheral nerve sheaths	Peripheral neuropathies	Sensorimotor neuropathies, painful neuropathies, ataxic gait, bilateral foot drop
Autoantibody activity to IgG	Cryoglobulinemia (type II)	Purpura, arthralgia, renal failure, sensorimotor neuropathies
Autoantibody activity to red blood cell antigens	Cold agglutinins	Hemolytic anemia, Raynaud phenomenon, acrocyanosis, livedo reticularis
Tissue deposition as amorphous aggregates	Organ dysfunction	Skin: bullous skin disease, papules, Schnitzler syndrome Gastrointestinal: diarrhea, malabsorption, bleeding Kidney: proteinuria, renal failure (light-chain component)
Tissue deposition as amyloid fibrils (light-chain component most commonly)	Organ dysfunction	Fatigue, weight loss, edema, hepatomegaly, macroglossia, organ dysfunction of involved organs (heart, kidney, liver, peripheral sensory and autonomic nerves)

Source: *Williams Hematology*, 9th ed, Chap. 109, Table 109–2.

The Hyperviscosity Syndrome

- Symptoms usually occur when the monoclonal IgM concentration exceeds 5 g/dL or when serum viscosity is more than 4.0 centipoises (cp) but can occur at lower serum concentrations of IgM.
- Presence of cryoglobulins contributes to increasing blood viscosity, as well as to the tendency to induce erythrocyte aggregation.
- Frequent symptoms are headache; impaired vision; mental status changes, such as confusion or dementia; altered consciousness that may progress to coma; ataxia; or nystagmus.
- Ophthalmoscopic changes include link-sausage appearance of retinal veins, retinal hemorrhages, and papilledema and/or distended and tortuous retinal veins, hemorrhages, and papilledema.
- Congestive heart failure may develop, particularly in the elderly.
- Inappropriate red cell transfusion can exacerbate hyperviscosity and may precipitate cardiac failure.

Cryoglobulinemia

- The monoclonal IgM can behave as a cryoglobulin (type I) in up to 20% of patients.
- Symptoms result from impaired blood flow in small vessels and include Raynaud phenomenon, acrocyanosis, and necrosis of the regions most exposed to cold, such as the tip of the nose, ears, fingers, and toes.

IgM-Related Neuropathy

- Peripheral neuropathy occurs in up to 40% of cases.
- The nerve damage is mediated by diverse pathogenetic mechanisms:
  — IgM antibody activity toward nerve constituents, causing demyelinating polyneuropathies
  — Endoneurial granulofibrillar deposits of IgM without antibody activity, associated with axonal polyneuropathy
  — Tubular deposits in the endoneurium, associated with IgM cryoglobulin
  — Amyloid deposits or neoplastic cell infiltration of nerve structures, which is less common
- Half of patients with IgM neuropathy may have a distinctive clinical syndrome that is associated with antibodies against a minor 100-kDa glycoprotein component of nerve, myelin-associated glycoprotein (MAG).
  — The anti–MAG related neuropathy is typically distal and symmetrical, affecting both motor and sensory functions; it is slowly progressive with a long period of stability.
  — Most patients present with sensory complaints, imbalance, and gait ataxia, owing to lack of proprioception; leg muscles atrophy in advanced stages.
- Patients with monoclonal IgM to gangliosides may have a demyelinating sensory neuropathy with chronic ataxic neuropathy, sometimes presenting with ophthalmoplegia.
  — Monoclonal IgM proteins that bind to gangliosides with a terminal trisaccharide moiety, including ganglioside M_2 (GM_2) and GalNac-GD1A, are associated with a chronic demyelinating neuropathy and severe sensory ataxia that are unresponsive to glucocorticoids.
- Anti-sulfatide monoclonal IgM proteins are associated with sensory-motor neuropathy.

- The POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, M protein, and skin changes) is rare in patients with WM.

Cold Agglutinin Hemolytic Anemia

- Monoclonal IgM may be cold agglutinins with binding activity for cell antigens at temperatures below 37°C, producing chronic hemolytic anemia.
  — This disorder occurs in less than 10% of WM patients.
  — It is associated with cold agglutinin titers greater than 1:1000 in most cases.
  — Mild to moderate chronic hemolytic anemia can be exacerbated after cold exposure.
  — The agglutination of red cells in the skin circulation also causes Raynaud syndrome, acrocyanosis, and livedo reticularis.

IgM Tissue Deposition

- The monoclonal protein can deposit in several tissues as amorphous aggregates.
- Amorphous deposits in the dermis are referred to as macroglobulinemia cutis.
- Deposition of monoclonal IgM in the lamina propria and/or submucosa of the intestine may be associated with diarrhea, malabsorption, and gastrointestinal bleeding.
- The incidence of cardiac and pulmonary involvement is higher in patients with monoclonal IgM than with other immunoglobulin isotypes.

Manifestations Related to Tissue Infiltration by Neoplastic Cells

- Pulmonary involvement in the form of masses, nodules, diffuse infiltrate, or pleural effusions is uncommon; the overall incidence of pulmonary and pleural findings is approximately 4%.
- Malabsorption, diarrhea, bleeding, or gastrointestinal obstruction may indicate involvement of the gastrointestinal tract at the level of the stomach, duodenum, or small intestine.
- Skin
  — This can be the site of dense lymphoplasmacytic infiltrates, similar to that seen in the liver, spleen, and lymph nodes, forming cutaneous plaques and, rarely, nodules.
  — Chronic urticaria and IgM gammopathy are the two cardinal features of the Schnitzler syndrome, which is not usually associated initially with clinical features of WM, although evolution to WM is not uncommon.

LABORATORY FINDINGS

- Anemia is the most common finding.
- Normocytic and normochromic anemia is present and rouleaux formation is often pronounced (**Figure 69–1**).
- Hemoglobin estimate can be inaccurate.
- Leukocyte and platelet counts are usually within the reference range at presentation.
- A raised erythrocyte sedimentation rate is almost always present.
- Thrombin time is often prolonged, and the prothrombin time and activated partial thromboplastin time may be prolonged.

- Serum monoclonal IgM protein level is high and typically ranges from 1.5 to 4.5 g/dL; serum levels of IgG and IgA are normal or low.

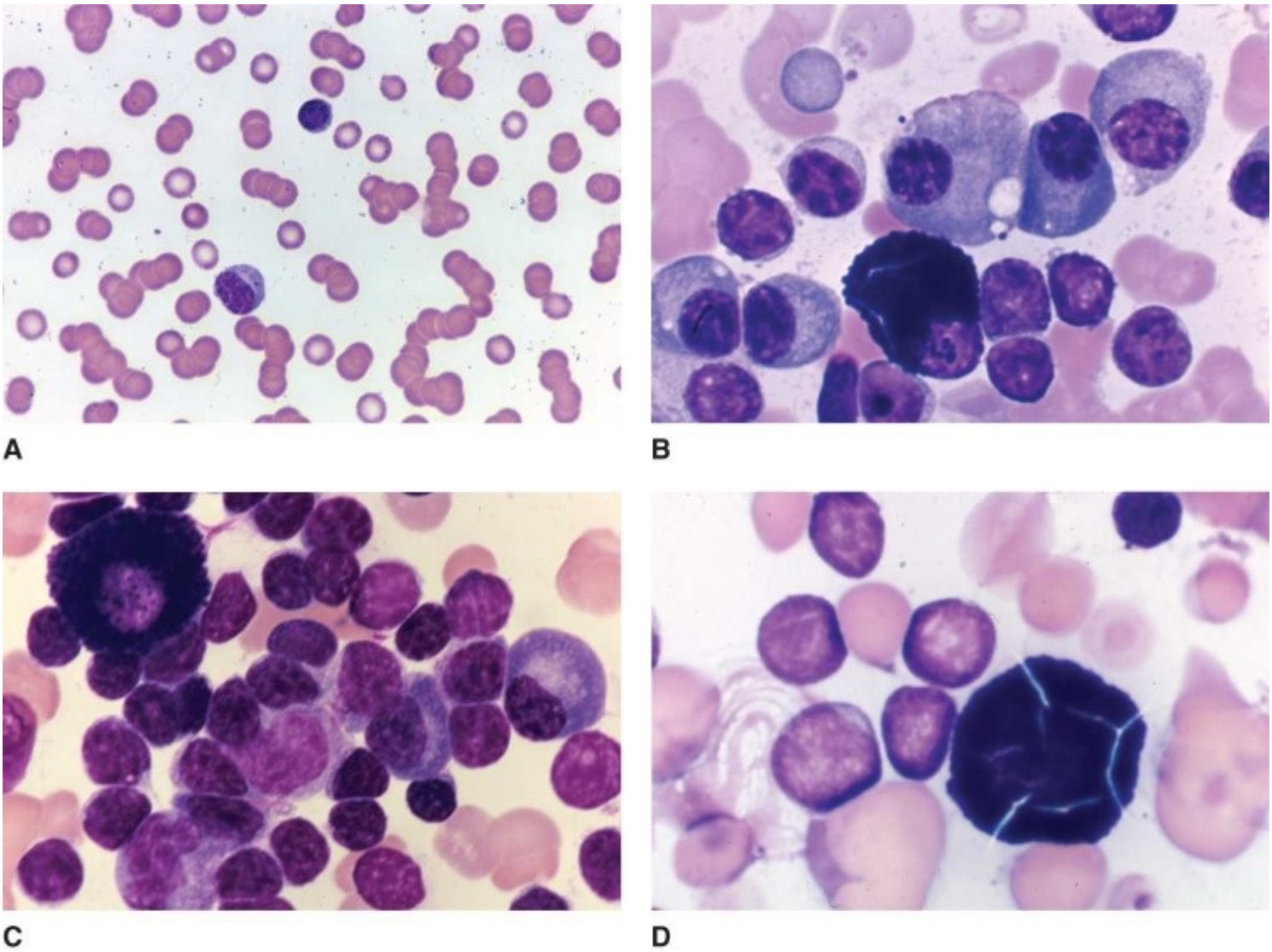


FIGURE 69–1 Waldenström macroglobulinemia. **A.** Blood film displaying the characteristic pathologic rouleaux seen as a result of the red cell aggregating properties of immunoglobulin M. **B.** Marrow film showing characteristic infiltrate of lymphocytes, lymphoplasmacytic cells, and plasma cells. A mast cell is evident lower center. Although not specific for this disease, mast cells are commonly present in the marrow. **C.** Marrow film showing infiltrate of lymphocytes with occasional plasma cells and a mast cell. **D.** Marrow film showing lymphocytic infiltrate with a "cracked" mast cell sometimes seen in this disease. The fraction of plasma cells varies as shown by the somewhat higher proportion in **(B)** as compared to **(C)** and **(D)**. Lymphocytes and lymphoplasmacytic cells predominate. (Reproduced with permission from *Lichtman's Atlas of Hematology*, www.accessmedicine.com.)

Marrow Findings

- The marrow is hypercellular, with diffuse infiltration of lymphocytes, plasmacytoid lymphocytes, and plasma cells (see **Figure 69–1**).
- It contains lymphoid cells with monoclonal surface membrane and/or cytoplasmic immunoglobulin.
- Increased numbers of mast cells admixed with aggregates of malignant lymphocytes are apparent.
- A solely paratrabecular pattern of lymphocyte infiltration is unusual and should raise the possibility of follicular lymphoma.

- The lymphocyte immunoprofile is Ig+CD19+CD20+CD22+CD79+.
- In up to 20% of cases, the lymphocytes may also express CD5, CD10, or CD23.

Immunologic Abnormalities

- High-resolution electrophoresis combined with immunofixation of serum and urine is recommended for identification and characterization of the IgM monoclonal protein.
- Testing for cold agglutinins and cryoglobulins should be performed at diagnosis.
  — If present, subsequent serum samples should be analyzed at 37°C for determination of serum monoclonal IgM level.
  — Although Bence Jones proteinuria is frequently present, it exceeds 1 g/24 h in only 3% of cases. Whereas IgM levels are elevated in WM patients, IgA and IgG levels are most often depressed and do not recover after successful treatment.

Serum Viscosity

- Serum viscosity should be measured if the patient has signs or symptoms of hyperviscosity syndrome.
- Among the first clinical signs of hyperviscosity are the appearance of peripheral and midperipheral dot and blot-like hemorrhages in the retina.
- In more severe cases of hyperviscosity, dot, blot, and flame-shaped hemorrhages can appear in the macular area along with markedly dilated and tortuous veins with focal constrictions resulting in “venous sausaging,” as well as papilledema.

RADIOLOGIC FINDINGS

- Marrow involvement can be documented by magnetic resonance imaging of the spine in more than 90% of patients.
- Computed tomography of the abdomen and pelvis demonstrates enlarged nodes in approximately 40% of WM patients.

TREATMENT

- Initiation of therapy should not be based on the IgM level per se, because this may not correlate with the clinical manifestations of WM.
- Initiation of therapy is appropriate for patients with constitutional symptoms, such as recurrent fever, night sweats, fatigue as a consequence of anemia, or weight loss.
  — Progressive symptomatic lymphadenopathy or splenomegaly provide additional reasons to begin therapy.
  — Anemia with a hemoglobin value of less than or equal to 10 g/dL or a platelet count of less than or equal to 100×10^9/L owing to marrow infiltration also justifies treatment.
- Plasmapheresis is used to help manage the hyperviscosity syndrome.

Initial Therapy

- Reasonable choices for the initial therapy of WM are alkylating agents (eg, chlorambucil), nucleoside analogs (cladribine or fludarabine), the monoclonal antibody rituximab, as well as combinations.
- Exposure to alkylating agents or nucleoside analogs should be minimized in patients who are candidates for autologous hematopoietic stem cell transplantation.

Oral Alkylating Agents

- Chlorambucil has been administered on both a continuous (ie, daily dose schedule) and an intermittent schedule.
  — Oral chlorambucil on a continuous schedule: orally 0.1 mg/kg per day
  — Oral chlorambucil on an intermittent schedule: orally 0.3 mg/kg per day for 7 days, every 6 weeks
- Median response duration was greater for patients receiving intermittent versus continuous-dose chlorambucil (46 vs 26 months).
- Chlorambucil (8 mg/m^2) plus prednisone (40 mg/m^2) given orally for 10 days, every 6 weeks, resulted in a major response (ie, reduction of IgM by > 50%) in 72% of patients.
- Pretreatment factors associated with shorter survival in the entire population of patients receiving single-agent chlorambucil are:
  — Age over 60 years, male sex, hemoglobin less than 10 g/dL, leukocytes less than 4×10^9/L, and platelets less than 150×10^9/L

Nucleoside Analog Therapy

- Cladribine, administered as a single agent by continuous intravenous infusion, by 2-hour daily infusion, or by subcutaneous bolus injections for 5 to 7 days, results in major responses in 40% to 90% of patients who received primary therapy, whereas in the previously treated patients, responses ranged from 38% to 54%.
- Fludarabine (25 mg/m^2 for 5 days) administered intravenously every 28 days to previously untreated or treated patients resulted in an overall response rate of 38% to 100% or 30% to 40%, respectively.
- Major toxicities of nucleoside analog therapy are myelosuppression and T-cell depletion, resulting in increased risk of opportunistic infections.
- Factors predicting a better response to nucleoside analogs:
  — Younger age at the start of treatment (< 70 years)
  — Higher pretreatment hemoglobin (> 9.5 g/dL)
  — Higher platelet count ($> 75 \times 10^9$/L)
  — Disease that does not relapse while on therapy
  — A long interval between first-line therapy and initiation of a nucleoside analog for relapsed disease
- Harvesting autologous peripheral blood stem cells succeeds on the first attempt in most patients who did not receive nucleoside analog therapy, compared with as few as one-third of patients who receive a nucleoside analog.

CD20-Directed Antibody Therapy

- Rituximab is a chimeric monoclonal antibody that targets CD20, a widely expressed antigen on lymphoplasmacytic cells in WM.
- Standard doses of rituximab (ie, four once-weekly infusions of 375 mg/m^2) induced major responses in approximately 30% of previously treated or untreated patients.
- The median time to treatment failure with rituximab ranged from 8 to over 27 months.
- A transient increase of serum IgM may be noted immediately following initiation of treatment with rituximab in many WM patients.
  - — The increase in IgM following initiation of therapy with rituximab does not portend treatment failure, and most patients return to their baseline IgM level by 12 weeks.
  - — Plasmapheresis should be considered in these patients in advance of rituximab therapy.
- Rituximab should not be used as sole therapy for the treatment of patients at risk for hyperviscosity symptoms.
- Time to response to rituximab therapy exceeds 3 months on the average.
- Patients with baseline serum IgM levels of less than 6.0 g/dL are more likely to respond.
- The objective response rate was significantly lower in patients who had either low serum albumin (< 3.5 g/dL) or a serum IgM monoclonal protein of more than 4.0 g/dL.
- Patients who had normal serum albumin and relatively low serum monoclonal protein levels derived a substantial benefit from rituximab, with a time to progression exceeding 40 months.

Proteasome Inhibitors

- Bortezomib is a proteasome inhibitor that induces apoptosis of primary WM lymphoplasmacytic cells.
  - — All but 1 of 27 patients with relapsed or refractory disease who received up to eight cycles of bortezomib at 1.3 mg/m^2 on days 1, 4, 8, and 11, had a response.
  - — The overall response rate was 85%, with 10 and 13 patients achieving a minor (> 25%) and major (> 50%) decrease in IgM level, respectively.
  - — Responses occurred at median of 1.4 months.
  - — The median time to progression for all responding patients was 7.9 (range: 3–21.4+) months.
  - — The most common grade III/IV toxicities were sensory neuropathies (22%), leukopenia (19%), neutropenia (15%), dizziness (11%), and thrombocytopenia (7%).
  - — Major responses occurred in 6 out of 10 (60%) previously treated patients.
- The combination of bortezomib, dexamethasone, and rituximab as primary therapy in patients with WM resulted in an overall response rate of 96%, and a major response rate of 83%.
  - — The incidence of grade 3 neuropathy was approximately 30% but was reversible in most patients following discontinuation of therapy.
- Carfilzomib has been evaluated in combination therapy.

Combination Therapies

- A regimen of rituximab, cladribine, and cyclophosphamide used in 17 previously untreated patients resulted in a partial response in approximately 95% of WM patients.
- The combination of rituximab and fludarabine led to an overall response rate of 95%, with

83% of patients achieving a major response.
— The median time to progression was 51 months.

- The combination of rituximab, dexamethasone, and cyclophosphamide achieved a major response in 74% of patients on this study, and the 2-year progression-free survival was 67%.
- The combination of cyclophosphamide, doxorubicin, vincristine, prednisone (CHOP) with rituximab (R-CHOP) has achieved major responses in approximately 80% to 90% of patients with relapsed or refractory disease.
- One study using two cycles of oral cyclophosphamide with subcutaneous cladribine as initial therapy reported a partial response in 84% of patients and the median duration of response of 36 months.
- A study evaluating fludarabine plus cyclophosphamide reported a response in 78% of patients and median time to treatment failure of 27 months.
- Various combination therapy regimens:
  — Nucleoside analogs and alkylating agents
  — Rituximab in combination with nucleoside analogs
  — Rituximab, nucleoside analogs, plus alkylating agents
  — Rituximab and cyclophosphamide-based therapy
  — Carfilzomib in combination with rituximab and dexamethasone

Novel Therapeutics

- Ibrutinib, which targets Bruton's tyrosine kinase, is approved by the US Food and Drug Administration for the treatment of symptomatic patients. In a study of previously treated patients with WM, the overall response rate was about 90%.
- Everolimus is an oral inhibitor of the mammalian target of rapamycin pathway. An overall response rate of about 70% was reported in previously treated patients.

High-Dose Therapy and Hematopoietic Stem Cell Transplantation

- The largest experience of hematopoietic stem cell transplantation for patients with WM was reported by the European Bone Marrow Transplant Registry. For autologous transplant recipients, the 5-year progression-free and overall survival rates were approximately 40% and 70%, respectively. Nonrelapse mortality at 1 year was 4%.
- Chemosensitive disease and fewer number of prior lines of therapy at time of the autologous transplantation were the most important prognostic factors for progression-free and overall survival.
- Allogeneic transplant recipients who underwent myeloablative conditioning had 5-year progression-free and overall survivals of approximately 60%; 3-year nonrelapse mortality was approximately 30%.

RESPONSE CRITERIA IN WM (TABLE 69–2)

- Major responses include partial, very good partial, and complete responses.
- Minor response is based on at least 25% to 50% decrease in serum IgM level.
- An important concern with the use of IgM as a surrogate marker of disease is that it can

fluctuate, independent of extent of tumor cell killing, particularly with newer biologically targeted agents such as rituximab and bortezomib.

TABLE 69–2 SUMMARY OF CONSENSUS RESPONSE CRITERIA FOR WALDENSTRÖM MACROGLOBULINEMIA

Complete response	CR	Absence of serum monoclonal IgM protein by immunofixation. Normal serum IgM level. Complete resolution of extramedullary disease (ie, lymphadenopathy/splenomegaly) if present at baseline. Morphologically normal marrow aspirate and trephine biopsy.
Very good Partial response	VGPR	Monoclonal IgM protein is detectable. 90% reduction in serum IgM level from baseline, or normalization of serum IgM level. Complete resolution of extramedullary disease (ie, lymphadenopathy/splenomegaly) if present at baseline. No new signs or symptoms of active disease.
Partial response	PR	Monoclonal IgM protein is detectable. ≥ 50% but < 90% reduction in serum IgM level from baseline. Reduction in extramedullary disease (ie, lymphadenopathy/splenomegaly) if present at baseline. No new signs or symptoms of active disease.
Minor response	MR	Monoclonal IgM protein is detectable. ≥ 25% but < 50% reduction in serum IgM level from baseline. No new signs or symptoms of active disease.
Stable disease	SD	Monoclonal IgM protein is detectable. < 25% reduction and < 25% increase in serum IgM level from baseline. No progression in extramedullary disease (ie, lymphadenopathy/splenomegaly). No new signs or symptoms of active disease.
Progressive disease	PD	> 25% increase in serum IgM level from lowest nadir (requires confirmation) and/or progression in clinical features attributable the disease.

Reproduced with permission from Owen RG, Kyle RA, Stone MJ, et al: Response assessment in Waldenström macroglobulinaemia: update from the VIth International Workshop, *Br J Haematol.* 2013 Jan;160(2):171–176.

COURSE AND PROGNOSIS

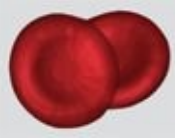
- **Table 69–3** lists several prognostic scoring systems that have been proposed for WM.
- Median duration of survival is 5 to 10 years.
- Major negative prognostic factors:
  - — Age older than 65 years
  - — Anemia less than 9 to 12 g/dL
  - — Thrombocytopenia (platelet count of < 100 to 150 × 10^9/L) or neutropenia (< 1.5 × 10^9/L)
  - — Elevated serum β_2-microglobulin levels (> 3.0 to 3.5 mg/L)
  - — Level of monoclonal IgM protein

TABLE 69–3 PROGNOSTIC SCORING SYSTEMS IN WALDENSTRÖM MACROGLOBULINEMIA

Study	Adverse Prognostic Factors	Number of Groups	Survival
Gobbi et al	Hgb < 9 g/dL	0–1 prognostic factors	Median: 48 months

	Age > 70 years Weight loss Cryoglobulinemia	2–4 prognostic factors	Median: 80 months
Morel et al	Age ≥ 65 years	0–1 prognostic factors	5-year: 87% of patients
	Albumin < 4 g/dL	2 prognostic factors	5-year: 62%
	Number of cytopenias: Hgb < 12 g/dL Platelets < 150×10^9/L WBC < 4×10^9/L	3–4 prognostic factors	5-year: 25%
Dhodapkar et al	β_2M ≥ 3 mg/L	β_2M < 3 mg/L + Hgb ≥ 12 g/dL	5-year: 87% of patients
	Hgb < 12 g/dL	β_2M < 3 mg/L + Hgb < 12 g/dL	5-year: 63%
	IgM < 4 g/dL	β_2M ≥ 3 mg/L + IgM ≥ 4 g/dL	5-year: 53%
		β_2M ≥ 3 mg/L + IgM < 4 g/dL	5-year: 21%
Application of International Staging System Criteria for Myeloma to WM (Dimopoulos et al)	Albumin ≤ 3.5 g/dL	Albumin ≥ 3.5 g/dL +β_2M < 3.5 mg/L	Median: NR
	β_2M ≥ 3.5 mg/L	Albumin ≤ 3.5 g/dL + β_2M < 3.5 or	Median: 116 months
		β_2M 3.5–5.5 mg/L	Median: 54 months
		β_2M > 5.5 mg/L	
International Prognostic Scoring System for WM (Morel et al)	Age > 65 years	0–1 prognostic factors (excluding age)	5 year: 87% of patients
	Hgb < 11.5 g/dL	2 prognostic factors (or age > 65 years)	5 year: 68%
	Platelets < 100×10^9/L	3–5 prognostic factors	5 year: 36%
	β_2M > 3 mg/L		
	IgM > 7 g/dL		

β_2M, β_2-microglobulin; Hgb, hemoglobin; NR, not reported; WBC, white blood cell count.
Source: *Williams Hematology,* 9th ed, Chap. 109, Table 109–4.



For a more detailed discussion, see Steven P. Treon, Jorge J. Castillo, Zachary R. Hunter, and Giampaolo Merlini: Macroglobulinemia, Chap. 109, in *Williams Hematology*, 9th ed.

CHAPTER 70
Heavy-Chain Diseases

DEFINITION

- The heavy-chain diseases (HCDs) are neoplastic disorders of B cells that produce monoclonal immunoglobulins (Ig) consisting of truncated heavy chains without attached light chains.
- In decreasing order of incidence, HCD involves synthesis of defective α, γ, or μ heavy chains.
- The diagnosis is established from immunofixation of serum, urine, or secretory fluids in the case of α-HCD or from immunohistologic analysis of the proliferating lymphoplasmacytic cells in nonsecretory disease.
- There is a high frequency of autoimmune disorders preceding or concurrent with the diagnosis of HCD, particularly γ-HCD.
- **Table 70–1** summarizes the clinical features of the three types of HCD.

TABLE 70–1 SUMMARY OF FEATURES OF THE HEAVY-CHAIN DISEASES

	Type of Heavy-Chain Disease		
Feature	**α**	**γ**	**μ**
Year described	1968	1964	1969
Incidence	Rare	Very rare	Very rare
Age at diagnosis	Young adult (< 30 years)	Older adult (60–70 years)	Older adult (50–60 years)
Demographics	Mediterranean region	Worldwide	Worldwide
Structurally abnormal monoclonal protein	IgA	IgG	IgM
MGUS phase	No	Rarely	Rarely
Urine monoclonal light chain	No	No	Yes
Urine abnormal heavy chain	Small amounts	Often present	Infrequent
Sites involved	Small intestine, mesenteric lymph nodes	Lymph nodes, marrow, spleen	Lymph nodes, marrow, liver, spleen
Pathology	Extranodal marginal zone lymphoma (MALT or IPSID)	Lymphoplasmacytoid lymphoma	Small lymphocytic lymphoma, CLL
Associated diseases	Infection, malabsorption	Autoimmune diseases	None
Therapy	Antibiotics, chemotherapy	Chemotherapy	Chemotherapy

CLL, chronic lymphocytic leukemia; Ig, immunoglobulin; IPSID, immunoproliferative small intestinal disease; MALT, mucosa-associated lymphoid tissue; MGUS, monoclonal gammopathy of undetermined significance.
Adapted with permission from Witzig TE, Wahner-Roedler DL: Heavy chain disease, *Curr Treat Options Oncol* 2002 Jun;3(3): 247–254.

ETIOLOGY AND PATHOGENESIS

- The etiology of γ-HCD and μ-HCD is unknown.
- In α-HCD, the lymphoplasmacytic infiltration of the intestinal mucosa is thought to be a response of the alimentary tract immune system to protracted luminal antigenic stimulation. A causal relationship between infection and pathogenesis is supported by a response to antibiotics in some cases.

CLINICAL AND LABORATORY FEATURES

γ-HCD

- Median age at presentation is in the late sixties.
- Clinical features are different than those of myeloma because renal disease and osteolytic lesions rarely occur.
- This form of HCD has various clinical and pathologic features that can be divided into three broad categories:
  — Disseminated lymphoproliferative disease: approximately 60% of patients
  — Localized proliferative disease: approximately 25% of patients
  — No apparent proliferative disease: approximately 15% of patients
- Most γ-HCD proteins are dimers of truncated heavy chains without associated light chains.
- The serum protein electrophoretic pattern is extremely variable, but a monoclonal peak is detected in over two-thirds of patients.
- The median value of the monoclonal spike at diagnosis in one study of 19 patients was 1.6 g/dL.
- The amount of HCD protein in the urine usually is small (< 1 g/24 h) but may reach 20 g/24 h.
- Patients commonly have moderate, normochromic, normocytic anemia.
- Autoimmune hemolytic anemia has been reported.
- Bone lesions are rare.

α-HCD

- This form of HCD defined by the recognition of truncated monoclonal α chains without associated light chains.
- Characteristic sharp spike of monoclonal gammopathy is not found on serum protein electrophoresis.
- Identification of the α-HCD protein depends on immunoselection or immunofixation.
- Majority of cases have been reported in northern Africa, Israel, and surrounding Middle Eastern countries.
- At presentation, the patients commonly are in their teens or early twenties.
- Common clinical features on presentation include recurrent or chronic diarrhea, weight loss, fevers, and/or growth retardation.
- Digital clubbing is a frequent finding.
- Moderate hepatomegaly occurs in about 25% of patients.
- Mesenteric lymphadenopathy is common, sometimes presenting as an abdominal mass,

whereas extra-abdominal lymphadenopathy is rare.
- In many cases, the abnormal heavy chain only can be found in the intestinal secretions.
- The jejunum is the usual site of involvement, with dense plasma-cell infiltration of the mucosa appearing during early stage disease (stage A). Infiltration of more blastic-appearing plasma cells is found extending beyond the lamina propria into the muscularis layer during later stages (stages B and C).
- Spread of neoplastic lymphoplasmacytic cell infiltration to mesenteric lymph nodes is characteristic of stage C disease.
- *Immunoproliferative small intestinal disease* is applied to small intestinal lesions with pathologic features identical to those of α-HCD regardless of the type of immunoglobulin synthesized.

μ-HCD

- Median age at presentation is in the late fifties.
- Infiltration of marrow with lymphocytes and plasma cells is common.
- Patients may have osteolytic lesions or pathologic fractures.
- Anemia is frequent.
- Lymphocytosis and thrombocytopenia are uncommon.
- Two-thirds of patients have monoclonal Ig light chains in the urine.
- Patients present symptoms of a lymphoproliferative malignancy (eg, chronic lymphocytic leukemia, B-cell lymphoma, Waldenström macroglobulinemia, or multiple myeloma).
- Diagnosis typically requires a combination or electrophoretic, immunoelectrophoretic, immunofixation, and immunophenotypic techniques.
- In a minority of cases, the Ig heavy chain can be identified by electrophoresis of serum or urine samples as a discrete homogenous band of β mobility.
- Immunoelectrophoresis and/or immunofixation are required to detect an Ig heavy-chain protein that does not react with either anti-κ or anti-λ antisera.
- Immunophenotypic analyses of biopsy material can reveal lymphoplasmacytic cells that stain positive for cytoplasmic Ig heavy chain, but not for Ig light chain.
- Bence Jones proteinuria is found in over half the cases of μ-HCD.
- Cases of nonsecretory μ-HCD have been reported.
- The presence of vacuolated plasma cells in the marrow of a patient with a lymphoplasmacytic proliferative disorder should always suggest the possibility of μ-HCD.

DIFFERENTIAL DIAGNOSIS

- All patients presenting with a lymphoplasmacytic cell proliferative disorder should be evaluated for γ-HCD and μ-HCD.
- The digestive form of α-HCD should be differentiated from other B-cell lymphomas.

TREATMENT, COURSE, AND PROGNOSIS

γ-HCD

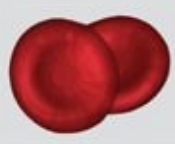
- Clinical course is variable and, thus, depends on the clinical features.
- Survival ranges from 1 month to over 20 years.
- Patients with lymphadenopathy on presentation have a more aggressive course than do patients with little evidence of lymphoproliferative disease.
- The amount of serum γ-HCD protein usually parallels the severity of the associated malignant disease.
- Disappearance of the monoclonal component from serum and urine associated with apparent complete response has been induced by chemotherapy, radiotherapy, or surgical removal of a localized lymphatic mass.
- In an asymptomatic patient, therapy is usually not necessary.
- In symptomatic patients with a low-grade lymphoplasmacytic malignancy, a trial of chlorambucil may be beneficial.
- Melphalan and prednisone can be used if the proliferation is predominantly plasmacytic.
- A trial of cyclophosphamide, vincristine, and prednisone with or without doxorubicin is reasonable for patients with evidence of a progressive lymphoplasmacytic cell proliferative process or high-grade B-cell lymphoma.
- Rituximab monotherapy was given in two cases, resulting in clinical responses in both.

α-HCD

- Clinical course is variable but generally progressive in the absence of therapy.
- Antibiotic therapy with tetracycline, metronidazole, or ampicillin is indicated for stage A disease patients who do not have parasitic infection.
  — Antibiotic therapy can result in complete response in 70% of patients.
- Patients with stage B or C disease or stage A lesions without improvement after a 60-month course of antibiotic treatment should be given chemotherapy. The treatment regimens are those commonly used to treat B-cell lymphoma (eg, R-CHOP).
- Surgical resection should be considered for focal or bulky transmural lymphomatous tumors in the gastrointestinal tract and extramedullary plasmacytoma.
- Autologous hematopoietic stem cell transplantation has been recommended for patients with advanced or refractory disease.

μ-HCD

- There is no specific therapy for μ-HCD.
- Chemotherapy is similar to that used in chronic lymphocytic leukemia (see Chap. 55) or in myeloma (see Chap. 68).
- Clinical course is variable, with survival ranging from 1 month to 11 years after appearance of symptoms.



For a detailed discussion, see Dietlind L. Wahner-Roedler and Robert A. Kyle: Heavy-Chain Disease, Chap. 110 in *Williams Hematology*, 9th ed.

CHAPTER 71
Amyloidosis

DEFINITION

- Amyloidosis is a heterogeneous group of diseases characterized by tissue infiltration with misfolded protein precursors.
- The term *amyloid* is used to describe a substance with a homogeneous eosinophilic appearance by light microscopy, a green birefringence on polarizing light microscopy, and a characteristic β-pleated sheet appearance by x-ray diffraction.
- Terms such as *primary, secondary, senile, dialysis-associated,* and *myeloma-associated* have been abandoned in favor of the etiologically based, chemical terminology (**Table 71–1**) (eg, immunoglobulin light chain amyloidosis is termed *AL amyloidosis*).

TABLE 71–1 NOMENCLATURE OF AMYLOIDOSIS

Amyloid Type	Subunit Protein	Clinical Organ Involvement
AL (κ or λ) or AH	Immunoglobulin light or heavy chain May be localized or systemic	Kidney Liver Heart Nerve
AA	Secondary serum amyloid A	Kidney Gastrointestinal Thyroid
ATTR (age related)	Senile systemic transthyretin	Heart Carpal tunnel
ATTR (mutant)	Familial transthyretin	Heart Nerve
A Lect-2	Leukocyte chemotactic factor No mutation found	Kidney
A Ins	Insulin localized to injection sites	Skin
A Fib	Fibrinogen A-2 mutation	Kidney
A β2M	β2-Microglobulin Chronic dialysis	Soft tissue Joints spine

Source: *Williams Hematology,* 9th ed, Chap. 108, Table 108–1.

EPIDEMIOLOGY

- The incidence of AL amyloidosis is rare, with an incidence of 8 per million persons per year and a median age at diagnosis of 67 years.
- Amyloid A (AA) amyloidosis is increasingly rare, occurring in less than 1% of persons with

chronic inflammatory diseases in the United States and Europe.

- AA amyloidosis is more common in Turkey and the Middle East, where it occurs in association with familial Mediterranean fever.
- AA is the only type of amyloidosis that occurs in children.
- Amyloid β_2-microglobulin ($A\beta_2M$) amyloidosis usually manifests as deposits in the joint synovial and occurs in patients on long-term dialysis. The incidence is declining with changes in dialysis techniques.
- The inherited amyloidoses are rare in the United States, with an estimated incidence of less than approximately 1 per 100,000 persons.
- Amyloidogenic transthyretin (ATTR) amyloidosis is the most common form of familial amyloidosis and is associated with mutations of the gene encoding transthyretin (TTR).

ETIOLOGY AND PATHOGENESIS

- The exact mechanism of fibril formation is unknown and may be different among the various types of amyloid.
- Amyloid precursor proteins typically consist of long fibrils composed of relatively small precursor proteins with molecular weights between 4000 and 25,000 daltons.
- Each amyloid fibril protein has a precursor molecule in the serum.
  — The secondary structures of many of the proteins have substantial β-pleated sheet structure. The known exceptions include serum amyloid A (SAA) and cellular prion protein (PrP^c), which contain little or no β folding in the precursor protein despite extensive β-sheet in the deposited fibrils.
- Amyloid formation involves:
  — Stimulus-generated change in the serum concentration or primary structure of amyloid precursor proteins.
  — Conversion of the precursor protein to amyloid fibrils.
- In some cases, the aberrant secondary structure seen in amyloid formation reflects a hereditary alteration in amino acid sequence that predisposes to fibril formation.
  — Examples include TTR, lysozyme, fibrinogen, cystatin c, gelsolin, amyloid-β protein precursor (AβPP), or apolipoprotein A-I (ApoA1).
- In other cases, wild-type molecules are the fibril precursor.
  — Examples include immunoglobulin light chain, β_2-microglobulin, ApoA1, and others.
- AL protein (immunoglobulin light chain):
  — AL amyloidosis is usually caused by a plasma cell neoplasm in the marrow and can occur in isolation or along with myeloma (see Chap. 68).
  — Marrow fibril deposits are composed of intact 23-kDa monoclonal immunoglobulin light chains.
  — Although both kappa (κ) and lambda (λ) light-chain subtypes have been identified in amyloid fibrils, λ subtypes predominate.
  — λ VI subtype appears to have unique structural properties that predispose it to fibril formation, often in the kidney.
- AL amyloidosis can be seen in other B lymphoproliferative disorders, including

macroglobulinemia and other types of lymphoma.

- AL amyloidosis should be distinguished from nonamyloid monoclonal immunoglobulin deposition disease, in which the deposited immunoglobulin consists of both heavy and light chains, of which κ chains predominate.
- AA proteins (amyloid A proteins):
  — AA amyloidosis occurs in response to inflammation and as familial periodic and Mediterranean fever syndromes.
  — The AA amyloid fibrils are usually composed of an 8-kDa, 76-amino-acid amino-terminal portion of the 12-kDa precursor, SAA.
  — SAA is a polymorphic protein encoded by a family of active serum amyloid A (SAA) genes, which are acute phase apoproteins synthesized in the liver and transported by high-density lipoprotein (HDL3) in the plasma.
  — Years of an underlying inflammatory disease causing an elevated SAA usually precedes fibril formation.
- ATTR proteins (transthyretin):
  — These proteins occur in several familial syndromes and in senile amyloid.
  — ATTR amyloidosis also is known as familial amyloidotic polyneuropathy or cardiomyopathy.
  — Variant TTR molecules are prone to dissociation from stable tetramers and to unfolding, leading to misfolding, polymerization, and fibril formation.
  — Evidence of an age-related "trigger" is that senile cardiac amyloidosis, caused by the deposition of fibrils derived from normal wild-type TTR, is exclusively a disease of older people.
- $A\beta_2M$ (β_2-microglobulin):
  — This occurs in chronic hemodialysis patients.
  — Carpal tunnel and joint synovial membrane involvement is common.
- AP protein (P component):
  — This is a minor protein component of amyloid deposits.
  — Intravenously injected purified P component preferentially binds to amyloid deposits.
  — This property has been exploited clinically, using radiolabeled P component, to localize and quantify the total body burden of amyloid in the so-called serum amyloid P (SAP) scan.
  — There is structural homology with C-reactive protein.
- Apo E allele (Apo E4):
  — This is strongly associated with Alzheimer disease.
- In some instances, the amyloid precursors undergo proteolysis, which may enhance the kinetics of folding into a prefibrillar structural intermediate.
- In some of the amyloidoses (eg, Aβ or AA), a normal proteolytic process may be disturbed, yielding a higher than normal concentration of a prefibrillar molecule.

CLINICAL FEATURES

- The clinical manifestations of amyloidosis vary widely and arise because of amyloid deposition and interference with normal organ function.

- Common presenting symptoms and signs are:
  — Weakness and weight loss
  — Purpura, particularly in loose facial tissue (**Figure 71–1**)
  — Symptoms and physical findings that reflect the extent of organ dysfunction because of amyloid involvement

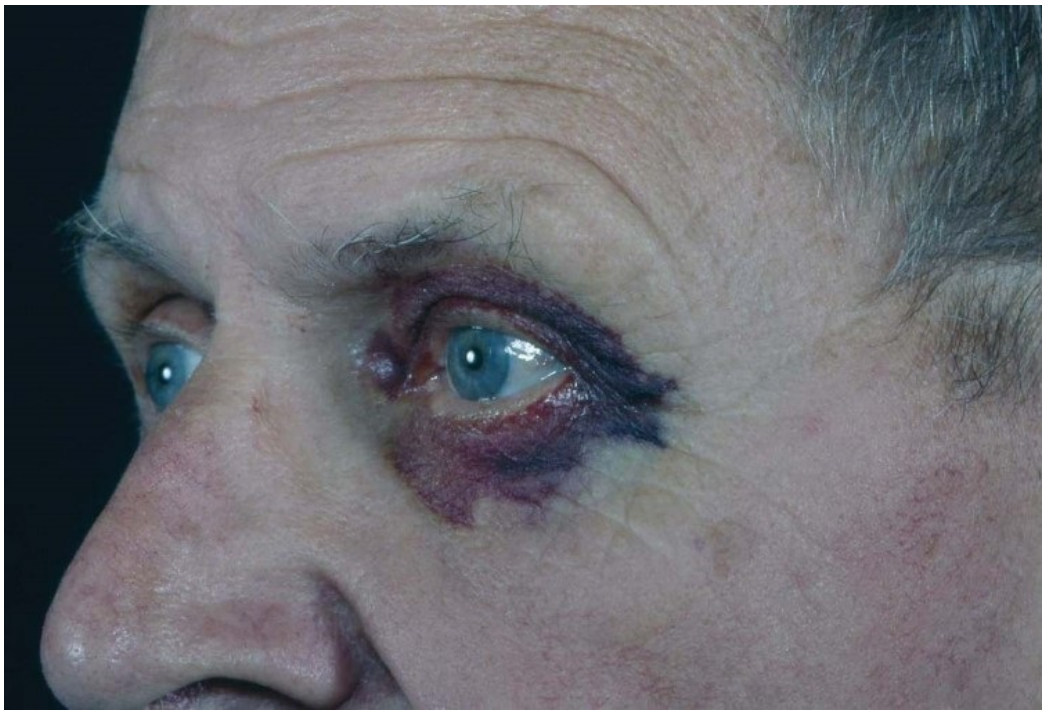


FIGURE 71–1 Amyloid purpura. Although periorbital purpura is uncommon, it is pathogenetic of AL amyloid. (Source: *Williams Hematology*, 9th ed, Chap. 108, Fig. 108–1.)

AL Amyloidosis

- **AL amyloidosis affects several organs.**
- Kidney:
  — Nephrotic syndrome and renal insufficiency
  — In a small proportion of patients (~10%), amyloid deposition in the renal vasculature or tubulointerstitium. This causes renal dysfunction without significant proteinuria.
- Liver and spleen:
  — Organ enlargement, hepatic cholestasis, and rarely traumatic rupture of enlarged spleen.
  — Profound elevation of alkaline phosphatase with only mild elevation of transaminase. This is characteristic of hepatic amyloidosis.
- Gastrointestinal tract:
  — Macroglossia, obstruction, ulceration, hemorrhage, malabsorption, and/or diarrhea
- Heart:
  — Congestive heart failure, cardiomegaly, and/or arrhythmias
  — Low voltage R waves in the echocardiogram
  — Restrictive cardiomyopathy
- Skin:
  — Lesions ranging from papules to large nodules and purpura
- Nervous system:

— Peripheral neuropathies, postural hypotension (autonomic neuropathy)
- Blood:
  — Characteristic coagulopathy because of depletion of factor X. Other factor deficiencies (eg, fibrinogen and factor IX) are related to liver disease.
- Soft tissues:
  — Macroglossia, carpal tunnel syndrome, skin nodules, arthropathy, alopecia, nail dystrophy, submandibular gland enlargement, periorbital purpura, and hoarseness of voice

AA Amyloidosis

- This type of amyloidosis can occur at any age.
- Primary clinical manifestation is proteinuria and/or renal insufficiency.
- Hepatosplenomegaly occurs in association with chronic inflammatory disorders.
- Cardiomyopathy rarely occurs.
- With chronic inflammatory diseases, amyloid progression is slow and survival is often more than 10 years, particularly with treatment for end-stage renal disease.

$A\beta_2M$ Amyloidosis

- Several distinct rheumatologic conditions are observed in $A\beta_2M$ amyloidosis, including carpal tunnel syndrome, persistent joint effusions, spondyloarthropathy, and cystic bone lesions.
- Carpal tunnel syndrome usually produces the first symptom of disease.
- Persistent joint effusions accompanied by mild discomfort occur in up to 50% of patients on dialysis for more than 12 years.

ATTR Familial Amyloidosis

- Some clinical features are similar to AL amyloidosis.
- The TTR variant, Val-122-IIe, is a common allele in the African American population and is associated with cardiomyopathy.

DIAGNOSIS

- A tissue biopsy demonstrating amyloid fibrils is necessary for the diagnosis of amyloidosis.
  — Amyloidosis is diagnosed by demonstration of Congo red–binding material with characteristic apple-green fluorescence under polarized light microscopy in a biopsy specimen.
  — Subcutaneous fat aspiration and marrow biopsy will identify 80% to 90% of patients later found to have amyloid elsewhere (**Figure 71–2**). Rectal biopsies are no longer favored.
  — Biopsy of an organ with impaired function is a high-yield procedure.
- Identification of a plasma cell neoplasm distinguishes AL amyloidosis from other types of amyloidosis, but these are a minority of cases.
  — There may be an increased percentage of plasma cells in the marrow.
  — Serum free light chain assay is the most frequent abnormality and is found in approximately

90% of patients.
— A monoclonal serum protein by itself is not diagnostic of amyloidosis, because essential monoclonal gammopathy is common in older patients.

- Consider the diagnosis in patients with:
  — Unexplained kidney disease with nephrotic syndrome
  — Unexplained neuropathy, congestive heart failure, or malabsorption
- The cardinal finding in AL amyloidosis is a monoclonal immunoglobulin light chain.
- AA amyloidosis should be suspected in patients with renal amyloidosis and a chronic inflammatory condition or infection.
- The suggested laboratory and radiographic testing for a patient with amyloid is shown in **Table 71–2**.

FIGURE 71–2 Technique and results of subcutaneous fat aspiration. **A.** Procedural technique. **B.** Fat stained with Congo red. Note the preserved interstices of the fat cells. **C.** Viewed under polarized light to demonstrate green birefringence. (Source: *Williams Hematology*, 9th ed, Chap. 108, Fig. 108–3.)

TABLE 71–2 SUGGESTED TESTING OF A KNOWN AMYLOID PATIENT

If mass spectroscopy identifies light-chain amyloid, consider localized amyloidosis (bladder, larynx, skin, bronchi)
If systemic (visceral involvement), perform the following tests:

- Alkaline phosphatase
- Aspartate aminotransferase
- β2-Microglobulin
- Bilirubin
- Calcium
- Creatinine
- Glucose
- Complete blood count
- Immunoglobulin free light chains
- Immunofixation and electrophoresis
- Serum and 24-hour urine
- Quantitative immunoglobulins
- N-terminal probrain natriuretic peptide
- Troponin T
- Factor X level
- Chest x-ray
- Electrocardiogram
- Echocardiogram
- Doppler and strain imaging
- Creatinine clearance

If mass spectroscopy identifies transthyretin (TTR) amyloid, perform these tests:
Echocardiogram
Doppler and strain imaging
Familial amyloidosis genetic testing (mass spectroscopy of serum TTR; if abnormal, TTR gene sequencing)

Source: *Williams Hematology*, 9th ed, Chap. 108, Table 108–3.

OTHER FORMS OF AMYLOIDOSIS

- The hereditary renal amyloidoses (amyloidosis ApoA-I [AApoAI], ApoA-II [AApoAII], amyloidosis fibrinogen α-chain [AFib], and amyloidosis lysozyme [ALys]) can resemble AL amyloidosis with renal involvement.
- The clinical differentiation is suggested by the family history and immunohistologic staining of biopsy material with antibodies specific for candidate amyloid precursor proteins.

Amyloidoses Localized to the Central Nervous System

- AL amyloidosis deposits are rarely found in the central nervous system, although they may be found in the cerebral vessels.
- The primary central nervous system amyloidoses include amyloidosis cystatin C (ACys) and hereditary cerebral hemorrhage with amyloidosis-Icelandic type, in which the precursor is the protease inhibitor cystatin c.

Localized Light Chain Amyloidosis

- The tracheobronchial tree is the most common site of localized AL amyloidosis; it does not progress to systematic disease.

Other Localized Amyloidosis

- Atrial natriuretic factor amyloidosis (AANF) affects older persons, often with congestive heart failure.
- In calcitonin amyloid (ACal), the precursor protein is calcitonin.
- In pancreatic islet cell amyloid polypeptide amyloidosis (AIAPP), the precursor protein is a polypeptide (IAPP), also known as amylin.
- In prolactin amyloid (Apro), prolactin or its fragments are found in the pituitary amyloid.
- Three proteins (gelsolin, keratoepithelin, and lactoferrin) have been found in fibrils from patients with autosomal dominant corneal amyloidosis.
  — Medin, an integral fragment of lactadherin, which is produced in aortic smooth muscle cell, forms the amyloid seen in the aorta of all older humans.
  — Insulin has been found in fibrils at the site of insulin injection.
  — Cytokeratin has been found in amyloidosis localized to the skin.

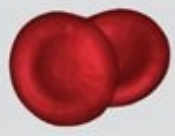
TREATMENT AND PROGNOSIS

- No specific treatment is available for amyloid disorders other than that caused by AL amyloidosis.

- Assessment of treatment response
  — Complete hematologic response is defined as absence of monoclonal protein in serum and urine by immunofixation electrophoresis, normal serum-free light-chain ratio and marrow biopsy with less than 5% plasma cells with no clonal predominance by immunohistochemistry.
- Prognosis (in AL amyloidosis)
  — Prognosis is determined by the extent of cardiac involvement using the degree of elevation of cardiac biomarkers (troponin T > 0.025 ng/mL; N-terminal pro-brain natriuretic peptide > 1800 pg/mL) and the plasma cell burden using the difference in serum free light chains (> 180 mg/L serum) as a quantitative reflection of plasma cell burden.
  — An elevation in each test is assigned 1 point. Four equal sized groups emerge: 0 points, 1 point, 2 points, and 3 points. The median survival for each group is 94, 40, 14, and 6 months, respectively.

AL Amyloidosis

- Melphalan and dexamethasone, which has supplanted melphalan and prednisone therapy, has a very low therapy-related mortality; 5-year actuarial survival may approach 50% but is highly dependent on the population treated.
  — Median responses as short as 11 to 18 months have been reported.
  — This therapy is considered the standard of care in patients who are not eligible for stem cell transplantation.
  — Clinically apparent cardiac involvement is an important adverse determinant of outcome.
- High-dose intravenous melphalan chemotherapy followed by autologous blood stem cell transplantation is presently considered the most effective treatment for patents with AL amyloidosis who do not have severe end-organ dysfunction (no more than 20% of patients will be eligible). The 10-year survival is approximately 40%.
- Melphalan, dexamethasone, and lenalidomide combination therapy has been reported for treatment of AL amyloidosis with overall response rates of about 60% and 2-year overall survival of 80%.
- Cyclophosphamide, thalidomide, and dexamethasone combination therapy has been reported to result in a 3-year estimated overall survival of about 80%.
- Bortezomib in combination with dexamethasone is effective, particularly in previously untreated patients; hematologic responses of 50% to 88% are seen.
  — This treatment has also been used as consolidation therapy following transplantation to deepen the response in patients who do not achieve a complete response.
- Supportive care to decrease symptoms and support organ function plays an important role in the management of this disease.



For a more detailed discussion, see Morie A. Gertz, Taimur Sher, Angela Dispenzieri, and Francis K. Buadi: Immunoglobulin Light Chain Amyloidoses, Chap. 108 in *Williams Hematology*, 9th ed.

PART IX

DISORDERS OF PLATELETS AND HEMOSTASIS

CHAPTER 72

Clinical Manifestations, Evaluation, and Classification of Disorders of Hemostasis

EVALUATION OF A SUSPECTED BLEEDING DISORDER

History

- A systematic approach is required to elicit and interpret all relevant information. Extensive, direct discussion between physician and patient is necessary to uncover the sometimes subtle details pertinent to a history of bleeding.
- Many otherwise healthy individuals, more often women than men, will report easy bruising and/or excessive bleeding.
- Patients with severe hemorrhagic disorders invariably have significantly abnormal histories of bleeding, either spontaneous or after trauma and/or interventions (eg, biopsies or surgical procedures).
- Typical clinical manifestations occur with specific hemostatic disorders, as outlined in **Table 72–1**.
- When evaluating the absence of prior bleeding, it is important to determine whether or not the patient has been exposed to significant hemostatic challenges, such as dental extraction, surgery, trauma, or childbirth.
- It is also important to attempt to obtain objective confirmation of the bleeding event and the severity, such as the need for blood transfusions, the development of anemia requiring iron replacement, hospitalization because of bleeding, ambulatory evaluation of a bleeding tendency, and the results of any laboratory studies done previously.
- A medication history is essential, with particular attention to nonprescription drugs (eg, aspirin or nonsteroidal anti-inflammatory drugs, or NSAIDs) and other drugs taken regularly and therefore easily forgotten, including herbal and other alternative medicines.
- A nutritional history is necessary to evaluate intake of vitamin K, vitamin C, and the adequacy of general nutrition.
- A history of bleeding involving one organ or system, such as hematuria, hematemesis, or hemoptysis suggests a local cause, such as a neoplasm. Bleeding from multiple sites may indicate a coagulation defect.
- Prolonged oozing of blood from sites of high fibrinolytic activity, such as the urinary tract, endometrium, or oral and nasal mucosa, may occur in patients with hemostatic abnormalities.
- Mucosal and cutaneous bleeding may also be caused by vascular disorders, such as hereditary hemorrhagic telangiectasia (Rendu-Osler-Weber syndrome) or scurvy (see Chap. 77).
- A detailed family history is particularly important, including all relatives going back at least one generation and specific inquiry about any consanguinity.
- A history of some bleeding problems may be suggestive of specific disorders.

- Epistaxis and gingival hemorrhage are the most common symptom of qualitative or quantitative platelet disorders, von Willebrand disease, and hereditary hemorrhagic telangiectasia.
- Cutaneous bruising may occur with a variety of hemostatic disorders.
- The frequency, size, location, color, and history of trauma are all relevant to evaluating the significance of the bruising.
- Dental extractions present severe hemostatic challenges that can be objectively evaluated by the need for suturing, packing, or transfusion.
- Prolonged bleeding from razor cuts inflicted while shaving often occur in patients with platelet disorders or von Willebrand disease.
- Hemoptysis, hematemesis, or hematuria is rarely the presenting symptom in patients with bleeding disorders.
- In patients who also have a local lesion, bleeding disorders may contribute to repeated episodes of hematochezia or melena.
- Menorrhagia occurs with platelet disorders and von Willebrand disease, but most often is not associated with a bleeding disorder.
- Excessive bleeding relating to pregnancy may occur with some bleeding disorders. Repeated spontaneous abortion may occur with factor XIII deficiency, hereditary disorders of fibrinogen, or the antiphospholipid syndrome.
- Hemarthroses occur with severe deficiencies of blood coagulation factors, especially the hemophilias, or with severe (type 3) von Willebrand disease.
- Bleeding after circumcision occurs with hemophilia A and B. Delayed bleeding after circumcision may be a result of factor XIII deficiency.
- Bleeding from the umbilical cord in newborns is a typical symptom of factor XIII deficiency.
- Prolonged bleeding from sites of venipuncture or other invasive procedures is typical of disseminated intravascular coagulation.

TABLE 72–1 CLINICAL MANIFESTATIONS TYPICALLY ASSOCIATED WITH SPECIFIC HEMOSTATIC DISORDERS

Clinical Manifestations	Hemostatic Disorders
Mucocutaneous bleeding	Thrombocytopenias, platelet dysfunction, von Willebrand disease
Cephalhematomas in newborns, hemarthroses, hematuria, and intramuscular, intracerebral, and retroperitoneal hemorrhages	Severe hemophilias A and B; severe deficiencies of factor VII, factor X, or factor XIII; severe type 3 von Willebrand disease; and afibrinogenemia
Injury-related bleeding and mild spontaneous bleeding	Mild and moderate hemophilias A and B; severe factor XI deficiency; moderate deficiencies of fibrinogen and factors II, V, VII, or X; combined factors V and VIII deficiency; and α_2-antiplasmin deficiency
Bleeding from stump of umbilical cord and habitual abortions	Afibrinogenemia, hypofibrinogenemia, dysfibrinogenemia, or factor XIII deficiency
Impaired wound healing	Factor XIII deficiency
Facial purpura in newborns	Glanzmann thrombasthenia, severe thrombocytopenia
Recurrent severe epistaxis and chronic iron-deficiency anemia	Hereditary hemorrhagic telangiectasias

Source: *Williams Hematology,* 9th ed, Chap. 116, Table 116–2.

Physical Examination

- Patients should be examined for petechiae, ecchymoses, telangiectases, and hematomas.
- Splenomegaly may occur in patients with thrombocytopenia.
- Venipuncture or other invasive sites should be examined for prolonged bleeding.
- Joints should be examined for deformity or restricted mobility.
- Throughout the examination, signs of underlying disorders that can cause hemostatic abnormalities should be sought (**Table 72–2**).

TABLE 72–2 CLASSIFICATION OF DISORDERS OF HEMOSTASIS

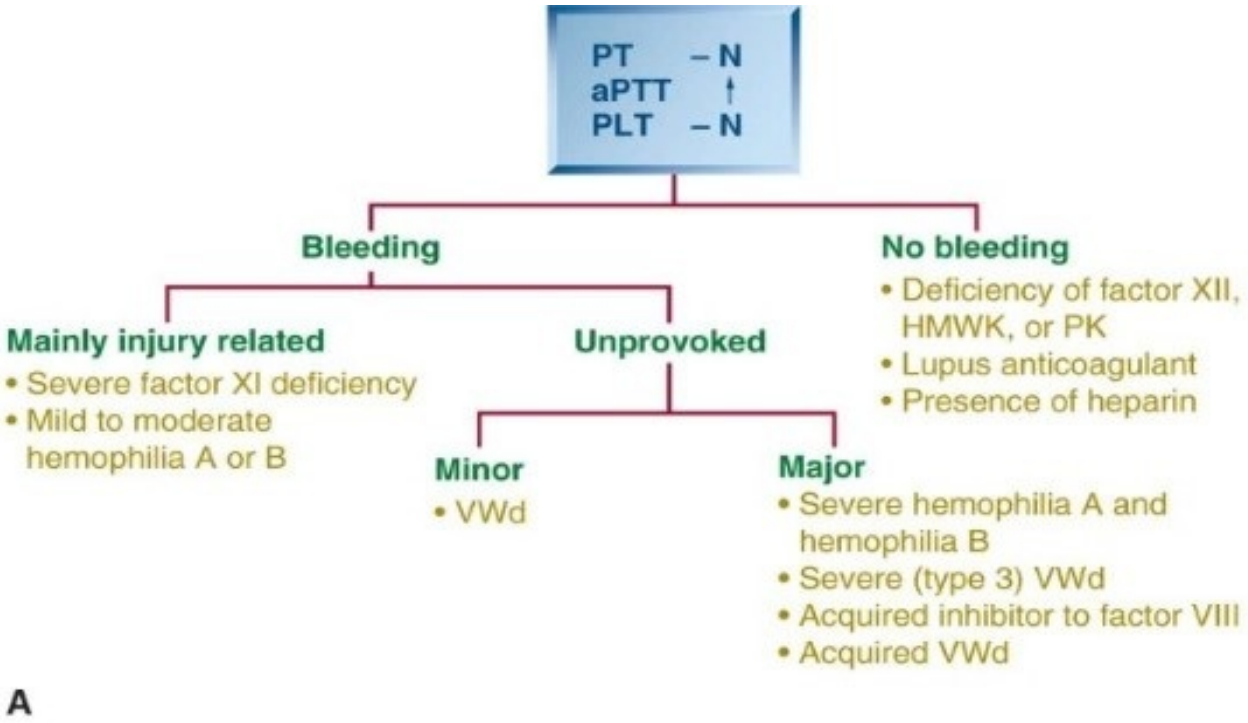
Major Types	Disorders	Examples
Acquired	Thrombocytopenias	Autoimmune and alloimmune, drug-induced, hypersplenism, hypoplastic (primary, suppressive, myelophthisic), DIC (see Chap. 73).
	Liver disease	Cirrhosis, acute hepatic failure, liver transplantation (see Chap. 83).
	Renal failure	Malabsorption syndrome, hemorrhagic disease of the newborn, prolonged antibiotic therapy, malnutrition, prolonged biliary obstruction (see Chap. 81).
	Vitamin K deficiency	
	Hematologic disorders	Acute leukemias (particularly promyelocytic), myelodysplasias, monoclonal gammopathies, essential thrombocythemia (see Chaps. 42, 44, 45, 54, and 67).
	Acquired antibodies against coagulation factors	Neutralizing antibodies against factors V, VIII, and XIII; accelerated clearance of antibody-factor complexes (eg, acquired von Willebrand disease, hypoprothrombinemia associated with antiphospholipid antibodies) (see Chaps. 82 and 84).
	Disseminated intravascular coagulation	Acute (sepsis, malignancies, trauma, obstetric complications) and chronic (malignancies, giant hemangiomas, missed abortion) (see Chap. 85).
	Drugs	Antiplatelet agents, anticoagulants, anti-thrombin agents, and thrombolytic, myelosuppressive, hepatotoxic, and nephrotoxic agents (see Chap. 87).
	Vascular	Nonpalpable purpura ("senile," solar, and factitious purpura), use of corticosteroids, vitamin C deficiency, child abuse, purpura fulminans; palpable purpura (Henoch-Schönlein, vasculitis, dysproteinemias) (see Chap. 79).
Inherited	Deficiencies of coagulation factors	Hemophilia A (factor VIII deficiency), hemophilia B (factor IX deficiency), deficiencies of factors II, V, VII, X, XI, and XIII and von Willebrand disease (see Chaps. 78, 79, and 81).
	Platelet disorders	Glanzmann thrombasthenia, Bernard-Soulier syndrome, platelet granule disorders, etc. (see Chap. 75).
	Fibrinolytic disorders	α_2-Antiplasmin deficiency, plasminogen activator inhibitor-1 deficiency (see Chap. 86).
	Vascular	
	Connective tissue disorders	Hemorrhagic telangiectasias (see Chap. 77). Ehlers-Danlos syndrome (see Chap. 77).

Source: *Williams Hematology,* 9th ed, Chap. 116, Table 116–1.

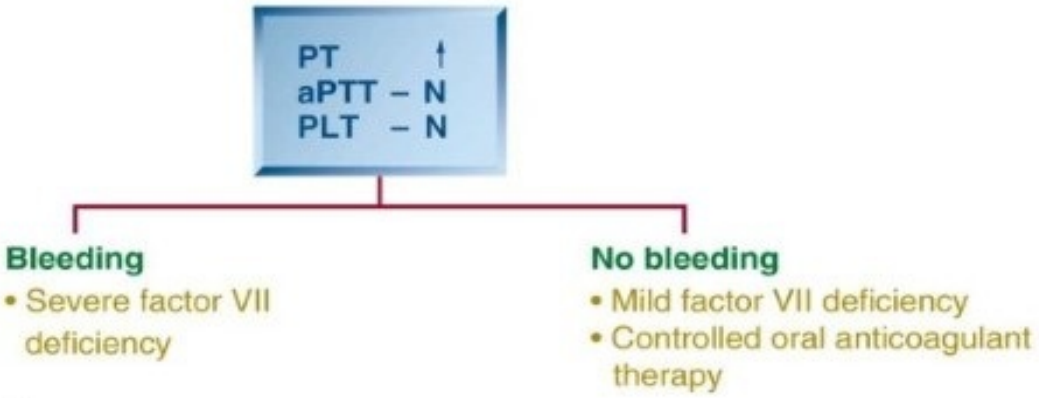
EVALUATION BASED ON HISTORY AND INITIAL HEMOSTATIC TESTS

Initial Hemostatic Tests

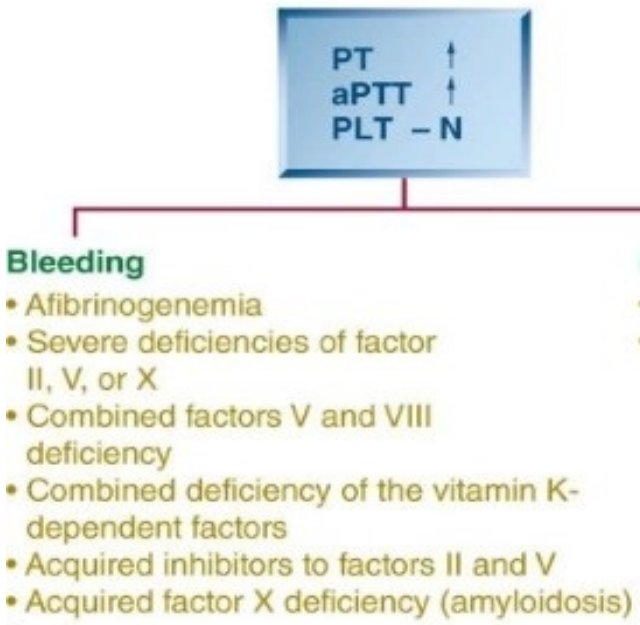
- The initial evaluation should include a prothrombin time (PT), activated partial thromboplastin time (aPTT), and a platelet count.
- In **Figure 72–1**, the results of these initial tests and the history of bleeding are integrated to suggest a tentative diagnosis of the hemostatic disorder.
- Prolongation of the PT, aPTT, or both may be a consequence of an inhibitor of one or more components of the coagulation scheme, as well as of a deficiency of an essential coagulation factor.
- It is possible to distinguish between an inhibiting antibody (inhibitor) and a deficiency by mixing equal parts of the patient's plasma with normal plasma, and repeating the test on the mixture. If a factor deficiency exists, the addition of normal plasma will lead to normal or nearly normal results, whereas if an inhibitor is present, the abnormality will persist.
- Some inhibitors, such as acquired antibodies to factor VIII, react slowly, and it is therefore necessary to incubate the mixture of normal and patient's plasma at 37°C for 2 hours before performing the coagulation assay.
- If the PT, aPTT, and platelet count are all normal, but the patient has a history of bleeding, platelet function tests, measurement of von Willebrand factor, factor XIII, and α_2-antiplasmin should be performed (**Figure 72–2**).
- Patients with mild types 1 or 2 von Willebrand disease may have sufficient factor VIII (> 30%) to give a normal aPTT, hence, direct measurement of von Willebrand factor activity is recommended.
- The thrombin time is prolonged by heparin; in disseminated intravascular coagulation; by an inhibitor present in plasma from patients with amyloidosis; and in patients with afibrinogenemia, hypofibrinogenemia, or dysfibrinogenemia.


PT – N
aPTT ↑
PLT – N
Bleeding
No bleeding
• Deficiency of factor XII, HMWK, or PK
• Lupus anticoagulant
• Presence of heparin
Mainly injury related
• Severe factor XI deficiency
• Mild to moderate hemophilia A or B
Unprovoked
Minor
• VWd
Major
• Severe hemophilia A and hemophilia B
• Severe (type 3) VWd
• Acquired inhibitor to factor VIII
• Acquired VWd

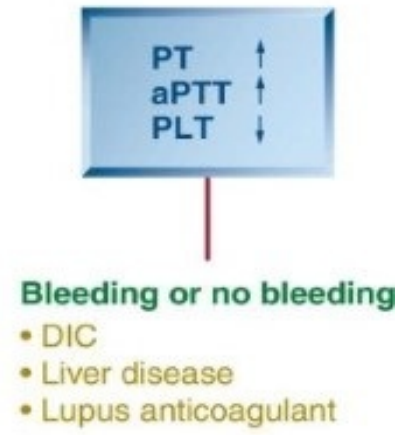
A


PT ↑
aPTT – N
PLT – N
Bleeding
• Severe factor VII deficiency
No bleeding
• Mild factor VII deficiency
• Controlled oral anticoagulant therapy

B


PT ↑
aPTT ↑
PLT – N
Bleeding
• Afibrinogenemia
• Severe deficiencies of factor II, V, or X
• Combined factors V and VIII deficiency
• Combined deficiency of the vitamin K-dependent factors
• Acquired inhibitors to factors II and V
• Acquired factor X deficiency (amyloidosis)
No bleeding
• Hypofibrinogenemia
• Mild deficiencies of factor II, V, or X

C


PT ↑
aPTT ↑
PLT ↓
Bleeding or no bleeding
• DIC
• Liver disease
• Lupus anticoagulant

D

FIGURE 72–1 Measures for establishing a tentative diagnosis of a hemostatic disorder using basic tests of hemostasis and the patient's history of bleeding. aPTT, activated partial thromboplastin time; DIC, disseminated intravascular coagulation; HMWK, high molecular weight kininogen; N, normal; PK, prekallikrein; PLT, platelets; PT, prothrombin time; VWd, von Willebrand disease. (Source: *Williams Hematology*, 9th ed, Chap. 116, Fig. 116–1.)

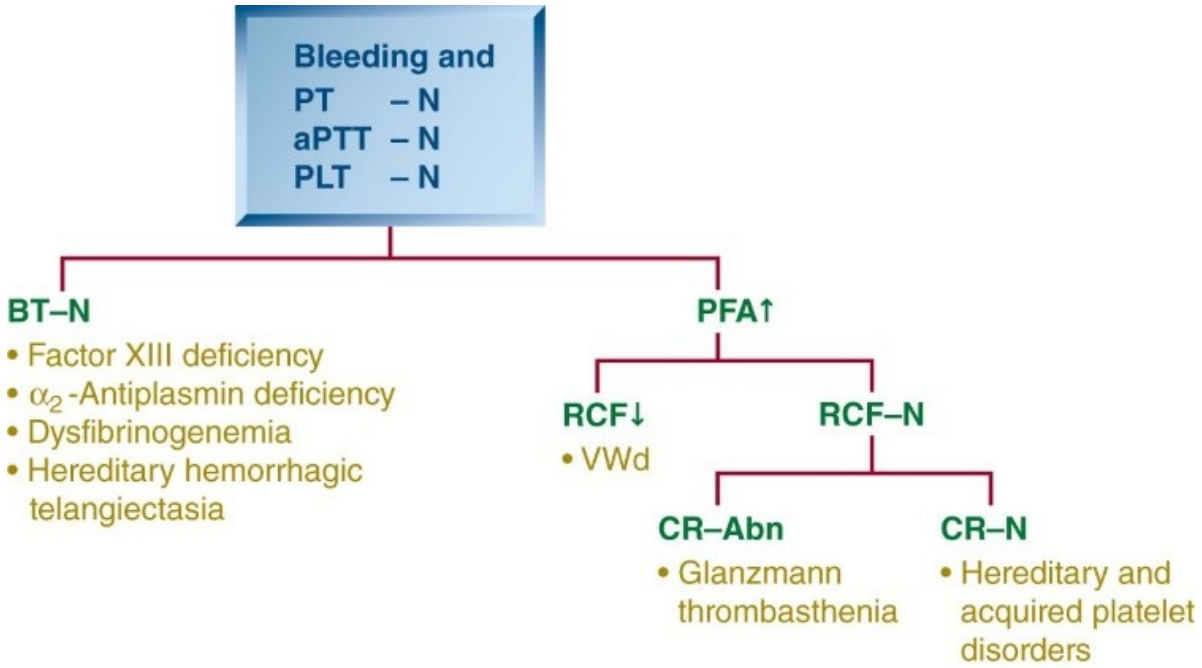


FIGURE 72–2 Tentative diagnoses in patients with bleeding manifestations and normal primary hemostatic tests using secondary tests. Abn, abnormal; aPTT, activated partial thromboplastin time; PFA, platelet function analyzer; CR, clot retraction; N, normal; PK, prekallikrein; PLT, platelets; PT, prothrombin time; RCF, ristocetin cofactor activity; VWd, von Willebrand disease. (Source: *Williams Hematology*, 9th ed, Chap. 116, Fig. 116–2.)

SPECIFIC ASSAYS FOR ESTABLISHING THE DIAGNOSIS

Thrombocytopenia (see also Chap. 73)

- It is essential to examine the blood film of all patients in whom a low platelet count is reported in order to rule out pseudothrombocytopenia. Alternatively, a platelet count in blood drawn in citrate can be performed.
- Examination of the blood film can also detect a number of abnormalities relevant to diagnosis of the cause of thrombocytopenia, as summarized in Table 72–3.

TABLE 72–3 CONDITIONS THAT MAY BE SUGGESTED BY EXAMINATION OF THE BLOOD FILM FROM PATIENTS WITH THROMBOCYTOPENIA

Disorder	Findings on Blood Film
Inherited thrombocytopenia	Giant platelets
May-Hegglin anomaly	Giant platelets and Döhle-like bodies in leukocytes
Diminished platelet survival (eg, idiopathic thrombocytopenic purpura)	Moderately enlarged platelets
Wiskott-Aldrich syndrome	Small platelets
Thrombotic microangiopathy (eg, thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, malignant hypertension), disseminated intravascular coagulation	Schistocytes, burr cells
Rouleaux formation	Dysproteinemia

Neutrophil hypersegmentation and macrocytosis	Vitamin B_{12} or folic acid deficiency
Abnormal leukocytes	Leukemia, myeloproliferative disorders

Factor Deficiencies (see also Chaps. 78 and 81)

- Modern clinical blood coagulation laboratories are capable of detecting deficiencies of specific coagulation factors.
- Immunologic techniques are available to determine whether the coagulation proteins are quantitatively decreased or qualitatively abnormal.

Inhibitors to Coagulation Factors

- Heparin does not require incubation to prolong the aPTT to a 1:1 mixture of patient's plasma and normal plasma. The presence of heparin in the plasma can be confirmed by finding a prolonged thrombin time that can be corrected by addition of toluidine blue, protamine, or other heparin inhibitors.
- Lupus-type anticoagulants are also active without incubation. Several methods are available for specific detection of lupus-type anticoagulants (see Chap. 84).
- Antibodies to specific coagulation factors, such as factor VIII, are usually detected only after incubation of a mixture of normal and patient's plasma for 2 hours at 37°C.
- Some inhibitors form complexes with specific coagulation factors in vivo. These are rapidly cleared from the circulation, and severe deficiency of the factor results. Special testing methods are required to detect such inhibitors.

Platelet Function Disorders

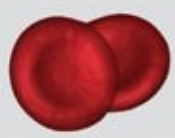
- A guide to the steps required for diagnosis of qualitative platelet disorders is given in Chaps. 75 and 76.

PREOPERATIVE ASSESSMENT OF HEMOSTASIS

- Preoperative assessment is based on the history of bleeding, any underlying disorder that compromises hemostasis, initial laboratory tests, and the type of surgery planned.

CLASSIFICATION OF HEMOSTATIC DISORDERS

- Hemostatic disorders can be conveniently classified as either hereditary or acquired, or according to the mechanism of the defect(s). **Table 72–2** classifies coagulation disorders as either "acquired" or "inherited."



For a more detailed discussion, see Marcel Levi, Uri Seligsohn, and Kenneth Kaushansky: Classification, Clinical Manifestations and Evaluation of Disorders of Hemostasis, Chap. 116 in *Williams Hematology*, 9th ed.

CHAPTER 73
Thrombocytopenia

- Thrombocytopenia is defined as a platelet count below the lower limit of normal for the specific method used (eg, <150 10^9/L).
- The types and causes of thrombocytopenia are listed in **Table 73–1**.

TABLE 73–1 CLASSIFICATION OF THROMBOCYTOPENIA

I. Pseudo (spurious)-thrombocytopenia
- A. Antibody-induced platelet aggregation
- B. Platelet satellitism
- C. Antiphospholipid antibodies
- D. Glycoprotein IIb/IIIa antagonists
- E. Miscellaneous

II. Thrombocytopenia resulting from impaired platelet production
- A. Inherited platelet disorders
- B. Acquired marrow disorders
  1. Nutritional deficiencies and alcohol-induced thrombocytopenia
  2. Clonal hematological diseases (myelodysplastic syndrome, leukemias, myeloma, lymphoma, paroxysmal nocturnal hemoglobinuria)
  3. Aplastic anemia
  4. Marrow metastasis by solid tumors
  5. Marrow infiltration by infectious agents (eg, HIV, tuberculosis, brucellosis)
  6. Hemophagocytosis
  7. Immune thrombocytopenia (ITP)
  8. Drug-induced thrombocytopenia
  9. Pregnancy-related thrombocytopenia

III. Thrombocytopenia resulting from increased platelet destruction
- A. Immune thrombocytopenia
  1. Autoimmune thrombocytopenia (primary and secondary ITP)
  2. Alloimmune thrombocytopenia
- B. Thrombotic microangiopathies (TTP, hemolytic uremic syndrome [HUS])
- C. Disseminated intravascular coagulopathy (DIC)
- D. Pregnancy-related thrombocytopenia
- E. Hemangiomas (Kasabach-Merritt phenomenon)
- F. Drug-induced immune thrombocytopenia (quinidine, heparin, abciximab)
- G. Artificial surfaces (hemodialysis, cardiopulmonary bypass, extracorporeal membrane oxygenation)
- H. Type 2B von Willebrand disease

IV. Thrombocytopenia resulting from abnormal distribution of the platelets
- A. Hypersplenism
- B. Hypothermia
- C. Massive blood transfusions
- D. Excessive fluid infusions

V. Miscellaneous Causes
- A. Cyclic thrombocytopenia, acquired pure megakaryocytic thrombocytopenia

Source: *Williams Hematology,* 9th ed, Chap. 117, Table 117–1.

SPURIOUS THROMBOCYTOPENIA (PSEUDOTHROMBOCYTOPENIA)

- A false diagnosis of thrombocytopenia can occur when laboratory conditions cause platelets to clump, resulting in artificially low platelet counts as determined by automated counters. This occurs in 0.1% to 0.2% of automated platelet counts. Occasionally, if a high proportion of platelets are unusually large, the automated count can be spuriously low.
- Blood films should always be carefully examined to confirm the presence of thrombocytopenia.

Etiology and Pathogenesis

- Falsely low platelet counts are caused by platelet clumping most often occurring in blood samples collected in EDTA anticoagulant. Blood collected in citrate will often confirm the spurious nature of the thrombocytopenia, although clumping may occur in any anticoagulant.
- Platelets may attach to each other to form clumps or may form clumps with leukocytes, usually neutrophils.
- Platelet clumping is usually caused by a low-titer IgG antibody reacting with an epitope exposed on platelet GP IIb/IIIa by in vitro conditions.

Laboratory Features

- A film made from blood anticoagulated with EDTA demonstrates more platelets than expected from the platelet count, but many are in large pools or clumps (see Figure 117–1 in *Williams Hematology*, 9th ed). A blood film made directly from a fingerstick sample accurately reflects the true count.
- Pseudothrombocytopenia is often accompanied by a falsely elevated white count because some platelet clumps are sufficiently large to be detected as leukocytes by an automated counter.
- Correct platelet counts can be obtained by placing fingerstick blood directly into diluting fluid at 37°C and performing counts by phase-contrast microscopy.

Clinical Features

- The platelet agglutinins causing spurious thrombocytopenia appear to have no other clinical significance.
- Platelet clumping is usually persistent.

THROMBOCYTOPENIA DUE TO SPLENIC POOLING (SEQUESTRATION) (SEE ALSO CHAP. 26)

Etiology and Pathogenesis

- The spleen normally sequesters about one-third of the platelet mass. Reversible pooling of up to 90% of the platelet mass occurs in patients with splenomegaly. A good example of this phenomenon is seen in patients with Gaucher disease.
- Total platelet mass is normal, platelet production is usually normal but may be reduced, and

platelet survival is often normal.
- Hypothermia can cause temporary thrombocytopenia in humans and animals, presumably because platelets are transiently sequestered in the spleen and other organs.

Clinical Features

- Thrombocytopenia caused by sequestration is often of no clinical importance. The degree of thrombocytopenia is moderate, the total body content of platelets is normal, and platelets can be mobilized from the spleen.
- In patients with liver disease and splenomegaly, bleeding is usually a result of blood coagulation disorders, and the thrombocytopenia is worsened by thrombopoietin (TPO) deficiency.
- Hepatic cirrhosis with portal hypertension and congestive splenomegaly is the most common disorder causing platelet sequestration, but any disease with an enlarged congested spleen can be associated with thrombocytopenia.
- The spleen is usually palpable, and the degree of thrombocytopenia is correlated with the size of the spleen.
- Patients with very large spleens and severe thrombocytopenia usually have decreased platelet production because of a marrow infiltrative process or severe liver disease, as well as sequestration.
- Only a few patients with hypothermia develop thrombocytopenia.

Laboratory Features

- Rarely is the platelet count less than 50×10^9/L unless a second contributing factor is present. Marrow megakaryocytes are usually normal in number and morphology.

Treatment and Prognosis

- Because thrombocytopenia caused by sequestration is usually not a clinically significant problem, no treatment is indicated.
- Splenectomy for another reason usually results in return of the platelet count to normal or above normal (see Chap. 26). Platelet counts may also return to normal after portal-systemic shunting for cirrhosis.
- Therapy for thrombocytopenia of hypothermia is rewarming and documenting normalization of platelet count.

THROMBOCYTOPENIA ASSOCIATED WITH MASSIVE TRANSFUSION

- Patients with massive blood loss requiring 15 or more units of red cells within 24 hours regularly develop thrombocytopenia with platelet counts as low as 25×10^9/L.
- The severity of the thrombocytopenia is related to the number of transfusions, but counts may be higher than predicted because of release from the splenic pool, or lower because of microvascular consumption.

- Management depends on the severity of the thrombocytopenia and the clinical condition of the patient.

HEREDITARY AND CONGENITAL THROMBOCYTOPENIAS

- These disorders generally have a clear inheritance pattern. Because prenatal infection or developmental abnormalities may be implicated, some are congenital but not hereditary.
- Thrombocytopenia may be the only abnormality, or it may be associated with well-defined abnormalities of platelet function, as in the Bernard-Soulier, Wiskott-Aldrich, and gray platelet syndromes (discussed in Chap. 75).
- Thrombocytopenia may be diagnosed at any age, including adulthood. In those cases discovered after infancy, a mistaken diagnosis of immune thrombocytopenia (ITP) may be made, particularly in children with moderate thrombocytopenia. Family studies can be helpful in such situations.

Fanconi Anemia (See Chap. 4)

- Autosomal recessive severe aplastic anemia usually beginning at age 8 to 9 years.
- Cells from homozygotes have increased sensitivity to chromosomal breakage by DNA cross-linking agents.
- Diverse congenital abnormalities may occur, including short stature, skin pigmentation, hypoplasia of the thumb and radius, and anomalies of the genitourinary, cardiac, and central nervous systems.
- Patients are at risk for acute leukemia and other malignancies.
- The condition is generally fatal unless corrected by allogeneic hematopoietic marrow transplantation with a reduced intensity conditioning regimen.

Thrombocytopenia with Absent Radius Syndrome

- Inheritance pattern suggests autosomal recessive but may be more complex.
- Usually noted at birth because of absence of both radii. Both ulnas are often absent or abnormal, and the humeri, bones of the shoulder girdle and feet may also be abnormal.
- One-third of patients have congenital heart anomalies.
- Allergy to cow's milk is common.
- Platelet counts are typically 15 to 30 × 10^9/L, lower during infancy and during periods of stress (surgery, infection). Thrombocytopenia may not be severe and may be overlooked until adulthood.
- Megakaryocytes are diminished or absent.
- Leukemoid reactions and eosinophilia are common.
- Treatments with glucocorticoids, splenectomy, and intravenous immunoglobulin (IVIG) are generally ineffective. Splenectomy may be effective in rare patients presenting as adults.
- Death is usually due to hemorrhage and usually occur within the first year.
- If patient can be sustained for the first 1 to 2 years of life, the platelet count usually recovers and survival is normal.
- Platelet counts vary during adulthood, but symptoms other than menorrhagia are unusual.

May-Hegglin Anomaly, Fechtner Syndrome, Sebastian Syndrome, and Epstein Syndrome

- May-Hegglin anomaly is characterized by autosomal dominant inheritance of giant platelets, and characteristic inclusion bodies in neutrophils, eosinophils, and monocytes. These resemble Döhle bodies seen with acute infections but have a different ultrastructure. Thrombocytopenia is common but may not be present and is rarely severe.
- Fechtner, Sebastian, and Epstein syndromes are quite similar to May-Hegglin anomaly but also manifest varying degrees of high-tone sensorineural deafness, nephritis, and cataracts.
- May-Hegglin anomaly, as well as Fechtner, Sebastian, and Epstein syndromes, are autosomal dominant macrothrombocytopenias with mutations in the *MYH9* gene, located on chromosome 22q12-13. This gene encodes nonmuscle myosin heavy chain (NMMHC)-IIA, which is expressed in platelets, kidney, leukocytes, and the cochlea.
- Platelets are large but ultrastructurally normal. Megakaryocytes are normal in appearance and number. Platelet survival and bleeding times are normal or slightly abnormal.
- The thrombocytopenia of most patients is well tolerated, and so usually no treatment is necessary, even for surgery or delivery, but platelet transfusions are commonly given.

X-linked Thrombocytopenia with Dyserythropoiesis

- A family of X-linked disorders of thrombocytopenia associated with dyserythropoiesis and thalassemia has been described, causing a modest bleeding diathesis proportionate to the degree of thrombocytopenia. These patients also have porphyria.
- GATA-1 is an erythroid and megakaryocyte specific transcription factor that drives gene expression essential for each of these two cell lineages.
- In several families, mutations in the amino terminal-finger are associated with macrothrombocytopenia and variable abnormalities in the erythroid lineage, whereas in other families mutations in the amino-terminal finger that disrupt the interaction of GATA-1 with a cofactor (FOG-1) lead to macrothrombocytopenia with dyserythropoietic anemia or β-thalassemia.
- Treatment is supportive, with platelet or erythrocyte transfusions if necessary.

Familial Platelet Syndrome with Predisposition to Myeloid Neoplasms

- Familial platelet syndrome with predisposition to acute myelogenous leukemia is a rare autosomal dominant condition characterized by qualitative and quantitative platelet defects resulting in pathologic bleeding and predisposition to the development of AML.
- Genetic analysis of several pedigrees linked the causative defect to a mutation in the transcription factor Runx-1 (also previously known as AML1 and CBFA2). Runx-1 binds to transcriptional complexes and regulates many genes important in hematopoiesis.
- Allogeneic hematopoietic stem cell transplantation is the only known, curative treatment.

Congenital Amegakaryocytic Thrombocytopenia

- Congenital amegakaryocytic thrombocytopenia (CAMT) is a rare autosomal recessive disease that in most cases presents with severe thrombocytopenia without physical abnormalities at birth.
- Bleeding complications usually are substantial because of the severe thrombocytopenia

present in these children.
- The disorder progresses to aplastic anemia before age 3 to 5 years in most patients.
- CAMT results from mutations in the gene encoding the TPO receptor *c-Mpl*, rendering it deficient (type I CAMT) or of reduced function (type II CAMT), or rarely due to mutation in the *TPO* gene.
- Treatment with allogeneic stem cell transplantation is essential for survival.

Thrombocytopenia with Radial-Ulnar Synostosis

- Patients with amegakaryocytic thrombocytopenia with radial-ulnar synostosis present at birth with severe normocytic thrombocytopenia with absent marrow megakaryocytes, proximal radioulnar synostosis, and other skeletal anomalies such as clinodactyly and shallow acetabulae.
- Bleeding complications are proportional to the degree of thrombocytopenia.
- Subsequent development of hypoplastic anemia and pancytopenia occur in several patients, suggesting that the defect is not limited to megakaryocytic progenitors.
- Genetic analysis of patients with thrombocytopenia and radioulnar synostosis revealed a mutation in *HoxA11*, known to be expressed in hematopoietic stem cells.

Wiskott-Aldrich Syndrome

- Wiskott-Aldrich syndrome (WAS) is a rare X-linked immunodeficiency disorder characterized by microthrombocytopenia, eczema, recurrent infections, T-cell deficiency, and increased risk of autoimmune and lymphoproliferative disorders (also see Chap. 50).
- The syndrome is caused by mutations of the *WASP* gene located on the short arm of the X chromosome (Xp11.22).
- The product of this gene, the WAS protein (WASP), is expressed in hematopoietic cells. WASP regulates actin polymerization and coordinates reorganization of the actin cytoskeleton and signal transduction pathways that occur during cell movement and cell–cell interaction.
- Supportive treatment during acute bleeding and disease complications consists of platelet transfusions, antibiotics, and systemic glucocorticoids when eczema is severe.
- Patients with mild phenotypes and severe thrombocytopenia may respond to splenectomy, but the risk of infection in these already immunocompromised patients may outweigh the benefit.
- If sufficiently severe, allogeneic hematopoietic stem cell transplantation is the only effective, curative treatment.

Paris-Trousseau Syndrome

- Paris-Trousseau syndrome and its variant Jacobsen syndrome are congenital dysmorphology syndromes in which affected individuals manifest trigonocephaly, facial dysmorphism, heart defects, and mental retardation.
- All affected patients have mild to moderate thrombocytopenia and dysfunctional platelets.
- The blood film shows a subpopulation of platelets containing giant α-granules. Marrow examination reveals two distinct subpopulations of megakaryocytes with expansion of immature megakaryocytic progenitors, dysmegakaryopoiesis, and many micromegakaryocytes.
- Pathologic bleeding usually is mild.

- Both disorders result from deletion of the long arm of chromosome 11 at 11q23, a region that includes the *FLI1* gene, the product of which is a transcription factor involved in megakaryopoiesis.
- The dominant inheritance pattern of Paris-Trousseau syndrome despite the presence of one normal allele seems to result from monoallelic expression of *FLI1* only during a brief window in megakaryocyte differentiation.

Autosomal Dominant Thrombocytopenia with Linkage to Chromosome 10

- This autosomal dominant thrombocytopenia displays variable degrees of thrombocytopenia, with bleeding proportionate to the degree of thrombocytopenia.
- Unlike familial platelet syndrome with predisposition to myeloid neoplasms, there is no risk of progression of the disease.
- Patients with this disorder have a genetic defect localized to 10p11-12 on the short arm of chromosome 10. In one large kindred with the disorder, a missense mutation was identified within the gene *FLJ14813*a, which encodes a putative tyrosine kinase of unknown function.
- Megakaryocyte precursors from affected individuals produce low numbers of polyploid cells in vitro, with delayed nuclear and cytoplasmic differentiation when analyzed by electron microscopy.

Kasabach-Merritt Syndrome

- Kasabach-Merritt syndrome is thrombocytopenia associated with giant cavernous angiomas. These lesions can infiltrate aggressively and require intensive treatment.
- The mechanism is platelet consumption in the tumor caused by intravascular coagulation.
- The hemangiomas are usually present at birth and neonatal thrombocytopenia may be present. The syndrome may develop in adults.
- Hemangiomas are usually solitary and superficial but may involve any internal organ.
- A bruit may be heard over the hemangioma, and cardiac failure may develop as a consequence of arteriovenous shunting.
- Thrombocytopenia may be severe, with marked red cell fragmentation. Laboratory abnormalities consistent with disseminated intravascular coagulation (DIC) are often present.
- Treatment may be necessary because of bleeding or growth of the tumor. Surgery can eliminate accessible lesions, and radiation therapy may be effective.
- In some cases, hemostatic abnormalities have been corrected by local thrombosis induced by antifibrinolytic agents, and thrombocytopenia has been corrected by treatment with antiplatelet agents.

ACQUIRED THROMBOCYTOPENIAS DUE TO DECREASED PLATELET PRODUCTION

- A heterogeneous group of disorders, including those caused by marrow aplasia (see Chap. 3), infiltration with neoplasms (Chap. 12), treatment with chemotherapeutic agents (see Chap. 38), and radiotherapy.

Megakaryocytic Aplasia

- Pure megakaryocytic aplasia or hypoplasia with no associated abnormalities is a rare disorder.
- Amegakaryocytic thrombocytopenia associated with other abnormalities such as dyserythropoiesis is more often seen and is likely a prodrome for myelodysplastic syndrome or aplastic anemia.
- Pure megakaryocytic aplasia appears to be a result of autoimmune suppression of megakaryocytes.
- The natural history is unclear and treatment with immunosuppression is empiric.

Infection

- Thrombocytopenia has been reported with diverse viral infections, usually the result of cytomegalovirus, Epstein-Barr virus, and hantavirus, in children receiving live-attenuated measles vaccine, and with many other infectious agents, such as *Mycoplasma, Plasmodium, Mycobacterium,* and *Ehrlichia.* The thrombocytopenia appears usually to be a result of decreased platelet production, but in some cases, immune-mediated platelet destruction may occur.

Thrombocytopenia Associated with Human Immunodeficiency Virus Infection

- Thrombocytopenia has been reported to occur in up to approximately 40% of adults with human immunodeficiency virus (HIV) infection but is usually of modest severity. It is usually not clinically relevant when the patient is successfully treated with highly active antiretroviral therapy, or HAART.

Etiology and Pathogenesis

- The principal cause is ineffective platelet production due to HIV infection of the stromal cells that facilitate hematopoiesis, such as macrophages and microvascular epithelial cells, and direct infection of megakaryocytes.
- Platelet survival is also decreased, possibly because of immune platelet injury.
- The occurrence of thrombocytopenia correlates with plasma viral load and CD4 cell depletion.
- Granulomatous infection or infiltration of the marrow with lymphoma may also contribute to the thrombocytopenia.

Clinical and Laboratory Features

- Platelet counts are rarely below 50×10^9/L, and thrombocytopenia frequently resolves spontaneously.
- The marrow contains normal or increased numbers of megakaryocytes and may be infiltrated with lymphoma or granulomas.

Treatment, Course, and Prognosis

- Antiretroviral drug regimens are the principal treatment for thrombocytopenia.

- Severe and symptomatic thrombocytopenia should be treated with prednisone (1 mg/kg per day) or with short courses of dexamethasone.
- IVIG given weekly at a dose of 0.4 g/kg for up to 5 weeks may be effective. Anti-D reagent has also been used.
- Splenectomy may be the most effective therapy, and does not appear to influence the course of the HIV infection adversely.

Nutritional Deficiencies and Alcohol-Induced Thrombocytopenia

Alcohol

- In alcoholics, thrombocytopenia is usually the result of cirrhosis with congestive splenomegaly, or of folic acid deficiency.
- Acute thrombocytopenia may also occur, because of direct suppression of platelet production by alcohol.
- After withdrawal of alcohol, platelet counts return to normal in 5 to 21 days and may rise above normal levels.

Nutritional Deficiencies

- Mild thrombocytopenia occurs in about 20% of patients with megaloblastic anemia caused by vitamin B_{12} deficiency. The frequency may be higher with folic acid deficiency because of the frequent association with alcoholism.
- Thrombocytopenia is caused primarily by ineffective platelet production.
- Iron deficiency typically causes thrombocytosis, but severe thrombocytopenia may occur, especially in children.

ACQUIRED THROMBOCYTOPENIA AS A RESULT PRIMARILY OF SHORTENED PLATELET SURVIVAL

Thrombotic Thrombocytopenic Purpura

- Thrombotic thrombocytopenic purpura (TTP) is a clinical syndrome of consumptive thrombocytopenia that left untreated results in a 95% mortality rate (see also Chap 90).

Etiology and Pathogenesis

- A well-documented mechanism for formation of platelet thrombi is disseminated platelet aggregation caused by increased plasma levels of ultra-high-molecular-weight multimers of von Willebrand factor (VWF). These appear to accumulate because of deficiency of a plasma VWF-cleaving metalloprotease (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 [ADAMTS13]).
- The deficiency may be inherited or more commonly acquired due to rapid clearance or inhibition of the enzyme by an autoantibody.

Clinical Features

- Sixty to 70% of patients with TTP are female.
- Full clinical expression of the disease is the "classic" pentad: thrombocytopenia, microangiopathic hemolytic anemia, neurologic symptoms, renal involvement, and fever.
- Because current treatment depends on prompt plasma exchange, the diagnosis now requires only thrombocytopenia and microangiopathic hemolytic anemia without another clinically apparent cause. However, more than 50% of patients also have neurologic signs and renal abnormalities.
- The most common presenting symptoms are neurologic abnormalities (headache, confusion, seizure, dysphagia, paresis), hemorrhage (epistaxis, hematuria, gastrointestinal bleeding, menorrhagia), fatigue, and abdominal pain.

Laboratory Findings

- Thrombocytopenia is essential for the diagnosis and is usually found at presentation or develops rapidly thereafter.
- Anemia and red cell fragmentation may also be absent at presentation but develop rapidly during the course of the disease.
- Consistent with severe hemolysis, serum lactic acid dehydrogenase (LDH) values are often markedly elevated and serum indirect bilirubin levels are also increased.
- Most patients have microscopic hematuria and proteinuria; some have acute, oliguric renal failure.
- Tissue biopsy is usually not required for diagnosis but may be necessary in difficult cases. The characteristic lesions are arteriolar and capillary thrombi composed primarily of platelets but also containing VWF and fibrin. Morphologically identical lesions are found in preeclampsia, malignant hypertension, acute scleroderma, and renal allograft rejection.
- Based on increased understanding of pathophysiology, several tests of ADAMTS13 activity are available. Severe acquired ADAMTS13 deficiency appears to be specific for TTP, although the sensitivity of the association is debated and the frequency of severe ADAMTS13 deficiency in TTP depends on how patients are ascertained. If adult patients with thrombotic microangiopathy are selected with no plausible secondary cause, no diarrheal prodrome, and no features suggestive of hemolytic uremic syndrome (HUS; eg, oliguria, severe hypertension, need for dialysis, serum creatinine > 3.5 mg/dL), then at least 80% have undetectable ADAMTS13 activity and the majority will have easily detected autoantibodies that inhibit the protease.

Differential Diagnosis

- Sepsis and DIC may cause an acute illness with fever, chills, and multiple organ dysfunction. The distinction should be clear from coagulation studies, which in TTP are not usually severely abnormal.
- Bacterial endocarditis can present with anemia, thrombocytopenia, fever, neurologic symptoms, and renal abnormalities.
- Evans syndrome, a combination of autoimmune hemolytic anemia and ITP, may be confused with TTP. The direct red cell antiglobulin (Coombs) test is usually positive in Evans syndrome.

- Other considerations include systemic lupus erythematosus, catastrophic antiphospholipid syndrome, scleroderma, megaloblastic anemia, or myelodysplastic thrombocytopenia.

Treatment

- Plasma exchange is the most important treatment modality.
- Rapid initial therapy with plasma exchange is essential. If facilities are not immediately available for apheresis, plasma infusions should be administered until the patient can be transferred to a facility that provides plasma exchange therapy.
- Plasma exchange is effective because of removal of the autoantibody and of large VWF multimers, and because of replacement of the ADAMTS13.
- Daily exchange of one plasma volume (40 mL/kg) is performed until the patient responds, as defined by correction of neurologic abnormalities, return to a normal platelet count, and normal or nearly normal serum LDH levels.
- Initial response typically occurs in the first week, and recovery is nearly complete in 3 weeks, but response may not occur for more than 1 month.
- If prompt response is not achieved, plasma exchange of 40 mL/kg should be done twice daily.
- After neurologic findings have resolved and the platelet count is normal, plasma exchange should be continued at increasing time intervals for another 1 to 2 weeks to avoid relapse, although solid evidence that such tapering of therapy reduces relapses is lacking.
- Renal function recovers more slowly than the neurologic and hematologic abnormalities. It is unknown if continued plasma exchange will affect recovery of renal function.
- With plasma exchange, mortality has been reduced from greater than 90% to less than 20%.
- With the realization that TTP represents an autoimmune disorder, treatment with glucocorticoids or other immunosuppressive regiments, such as rituximab, are appropriate and reduce relapse rates.
- Therapy with antiplatelet agents has not been generally effective and carries a significant risk of hemorrhage.
- In patients who have had a stroke or transient cerebral ischemic events, aspirin therapy is appropriate when severe thrombocytopenia has resolved.
- Anecdotal reports of success have appeared for numerous agents, including IVIG, vincristine, azathioprine, cyclophosphamide, cyclosporine, and extracorporal immunoadsorption.
- Platelet transfusion has been reported to exacerbate TTP and has been suggested as a cause of death in some reports.
- However, cautious administration of platelet transfusions may be indicated in some patients with major bleeding associated with marked thrombocytopenia.

Course and Prognosis

- Most of the now rare deaths occur early in the course of the disease.
- Approximately 30% to 50% of patients have relapsing disease.
- Patients who relapse long after the initial episode predictably recover with retreatment.
- It is not known whether or not those with chronic thrombocytopenia are more likely to relapse.
- The frequency of long-term sequelae after recovery from acute TTP is not known. Some patients may continue to have mild thrombocytopenia or abnormal renal function. Permanent

neurologic complications are uncommon.

Epidemic HUS in Children Caused by Shiga-Toxin–Producing *Escherichia coli*

- This type of HUS follows acute enteric infection with *E coli* (most often infection with *E coli* serotype 0157:H7) or *Shigella dysenteriae* serotypes, which produce the Shiga toxin.
- Progression of *E coli* 0157:H7 infection to HUS occurs in 2% to 7% of sporadic cases and in up to 30% of cases in some epidemics.
- Boys and girls are equally affected, and most cases occur between April and September.
- Most outbreaks are caused by undercooked beef, but other sources have been implicated including lettuce. Person-to-person transmission may also occur.

Clinical and Laboratory Features

- The major presenting symptom is diarrhea, which is bloody in most patients. The diarrhea may be sufficiently severe to require colectomy.
- Most patients are oliguric on admission, but average duration of symptoms before diagnosis of HUS is 6 days.
- Fever and hypertension are common. Pancreatitis and seizures may occur.
- Laboratory features are thrombocytopenia, microangiopathic hemolytic anemia, and the findings of acute renal failure.

Treatment, Course, and Prognosis

- Treatment is supportive. Approximately 50% of patients require dialysis.
- Plasma exchange appears to have minimal or no benefit.
- Mortality of epidemic childhood HUS is 3% to 10%, but HUS in the elderly may have mortality up to approximately 90%.
- Patients frequently have permanently impaired renal function after recovery.
- Relapses do not appear to occur.

TTP-HUS Associated with Infections Other than Shiga-Toxin–Producing *E coli*

- A syndrome resembling TTP and HUS has been reported to occur sporadically after infection caused by rickettsia, viruses, or bacteria other than those producing Shiga toxin.
- None of these infections is as clearly associated with TTP-HUS as is infection with *E coli* 0157:H7.
- It appears some infections may cause bona fide HUS; others may exacerbate existing TTP.
- Patients infected with HIV may develop a syndrome similar to TTP and HUS, but differ in having a gradual onset and a less predictable response to plasma exchange. Some patients survive for weeks or months without plasma exchange. These patients often have associated medical problems that could account for some of the findings interpreted as caused by TTP or HUS.

Drug-Induced TTP

- A syndrome resembling ADAMTS13-deficient TTP may be due to drug-dependent antibodies

to platelets and other cells.

Quinine

- Quinine is a frequent offender. Patients may have quinine-dependent antiplatelet antibodies. Some patients also have antineutrophil antibodies and develop severe neutropenia.
- Abdominal pain and nausea are common presenting symptoms.
- Plasma exchange is ineffective. Most patients also require hemodialysis, but they usually recover normal renal function.
- Reexposure to quinine, even in small amounts, can cause immediate recurrence.

Ticlopidine

- Acute, severe TTP-HUS has been reported to occur in some patients treated with short courses of ticlopidine.

Cancer Chemotherapy

- Nearly all chemotherapy patients who develop TTP have been treated with mitomycin C, most often for gastric cancer. Cisplatin, bleomycin, and pentostatin have also been reported to cause TTP.
- Induction of TTP by mitomycin C may be dose related, but less than 10% of patients receiving high doses develop the disease.
- Renal pathology is identical to that of other patients with TTP.
- The efficacy of plasma exchange is uncertain.
- Most patients die of their cancer or of renal failure.

Cyclosporine A

- A syndrome of severe renal failure, microangiopathic hemolytic anemia, and thrombocytopenia has been reported in patients receiving cyclosporine after allogeneic marrow transplantation, but the etiologic role of cyclosporine is uncertain.
- Tacrolimus has also been reported to cause TTP.

Other Drugs

- TTP has been associated with administration of metronidazole, cocaine, simvastatin, and ecstasy.

TTP Associated with Marrow Transplantation

- Most patients have had allogeneic transplants, but the disorder has also occurred with transplantation of autologous marrow or peripheral blood stem cells.
- The diagnosis of TTP may be difficult to establish because of the severe, multiorgan dysfunction accompanying allogeneic hematopoietic stem cell transplantation.
- All features of TTP in these patients could be caused by graft-versus-host disease, radiation toxicity, and systemic infection.
- Most patients do not respond to plasma exchange, and even in responsive patients plasma

exchange may not affect outcome.

TTP Associated with Cancer

- TTP may develop rarely in patients with metastatic carcinoma of various types, but more than half of such patients have had gastric cancer.
- Laboratory evidence of DIC is found in a minority of these patients.
- Therapy, course, and prognosis depend on the response of the tumor to chemotherapy. Plasma exchange appears not to be effective.

TTP Associated with Autoimmune Disorders

- Systemic lupus erythematosus, acute scleroderma, and the catastrophic antiphospholipid syndrome may present with clinical and pathologic findings difficult or impossible to distinguish from TTP.
- Treatment with plasma exchange has been reported to be effective in the severe autoimmune disorders as well as in TTP.

TTP Associated with Pregnancy

- TTP occurs in about 1 in 25,000 pregnancies.
- The clinical and pathologic features of TTP are similar to those of preeclampsia, particularly the HELLP syndrome (microangiopathic *h*emolytic anemia, *e*levated *l*iver enzymes, *l*ow *p*latelet count), suggesting a relationship between these disorders and complicating differential diagnosis.
- TTP has recurred in successive pregnancies in some patients and during pregnancy in women who have recovered from previous episodes of TTP unrelated to pregnancy. Pregnancy is therefore considered a risk for recurrence of TTP.
- With severe disease and a viable fetus, delivery should be induced. This will resolve preeclampsia but may or may not resolve platelet consumption. Some patients have delivered healthy term infants after developing TTP during the pregnancy.

THROMBOCYTOPENIA IN PREGNANCY

Gestational Thrombocytopenia

- Gestational thrombocytopenia, as defined by the following five criteria, occurs in approximately 5% of pregnancies: mild, asymptomatic thrombocytopenia; no past history of thrombocytopenia (except during a prior pregnancy); occurrence during late gestation; absence of fetal thrombocytopenia; and spontaneous resolution after delivery.
- Platelet counts are usually greater than 70×10^9/L and most are between 130 and 150×10^9/L. Lower platelet count or onset early in gestation suggest the diagnosis of immune thrombocytopenia (see “Immune Thrombocytopenia,” below).
- Usual obstetric care is appropriate for both mother and infant.

Preeclampsia

- Preeclampsia is defined by the presence of hypertension, proteinuria, and edema occurring during pregnancy and resolving after delivery. Eclampsia is preeclampsia plus neurologic abnormalities occurring peripartum.
- Thrombocytopenia develops in approximately 15% of women with preeclampsia, but platelet counts below 50×10^9/L occur in less than 5%.
- Some patients with severe preeclampsia may develop the HELLP syndrome, which may mimic TTP.
- Delivery of the fetus is the most effective approach to these disorders. Recovery usually begins promptly but may be delayed for several days.
- Plasma exchange is indicated for patients with severe thrombocytopenia and microangiopathic hemolytic anemia if the fetus cannot be delivered or prompt recovery does not occur after delivery. Earlier initiation of plasma exchange is indicated for severe clinical problems, such as acute, anuric renal failure or neurologic abnormalities.

IMMUNE THROMBOCYTOPENIA

- Immune thrombocytopenia (ITP) is an acquired disease of children and adults that is defined as isolated thrombocytopenia with no clinically apparent associated condition or other causes of thrombocytopenia. No specific criteria establish the diagnosis of ITP; the diagnosis relies on exclusion of other causes of thrombocytopenia.
- Adult ITP typically has an insidious onset and rarely resolves spontaneously.
- Childhood ITP characteristically is acute in onset and resolves spontaneously within 6 months.

Adult ITP

Etiology and Pathogenesis

- The mechanism of thrombocytopenia appears to be shortened intravascular survival of platelets as a consequence of splenic sequestration and destruction caused by antiplatelet antibodies.
- Antiplatelet antibodies also appear to bind to megakaryocytes and interfere with thrombopoiesis, leading to normal or decreased rates of platelet production even with increased or normal numbers of megakaryocytes in some patients.
- Most patients with ITP have demonstrable antibodies to platelet membrane glycoproteins GP IIb/GP IIIa and/or GP Ib/IX, but their specific pathogenetic role is not clear because they are also demonstrable in other conditions.
- In some patients, impaired platelet function is demonstrable, but its clinical significance is unknown.
- In some patients, it is likely that T-cell–mediated immune dysfunction is responsible for thrombocytopenia, and such patients are less likely to respond to now standard immunosuppressive treatments (rituximab, immune globulin).

Clinical Features

- Adult ITP appears to be more common in young women than in young men, but among older

patients, the sex incidence may be equal.

- Most adults present with a long history of purpura, but many patients are now asymptomatic at diagnosis because of the widespread inclusion of platelet enumeration in routine blood counts.
- Petechiae are not palpable, and they occur most often in dependent regions. Hemorrhagic bullae may appear on mucosal surfaces with severe thrombocytopenia.
- Purpura, menorrhagia, epistaxis, and gingival bleeding are common. Gastrointestinal bleeding and hematuria are less so. Intracerebral hemorrhage occurs in approximately 1% of patients but is the most common cause of death.
- Overt bleeding is rare unless thrombocytopenia is severe ($< 10 \times 10^9$/L), and even at this level most patients do not experience major hemorrhage.
- A palpable spleen strongly suggests that ITP is *not* the cause of thrombocytopenia.

Laboratory Features

- Thrombocytopenia is the essential abnormality. The blood films should be reviewed to rule out pseudothrombocytopenia (see above). The platelets are usually of normal size but may be enlarged.
- White blood cell count is usually normal, and the hemoglobin level is also normal unless significant hemorrhage has occurred.
- Coagulation studies are normal.
- The bleeding time does not provide useful information.
- Marrow megakaryocytes may be increased in number, with a shift to younger, less polypoid forms, but assessment of megakaryocyte morphology and number is not quantitative. In a patient with isolated thrombocytopenia and no symptoms or signs pointing to other causes a marrow examination is not necessary.

Differential Diagnosis

- The diagnosis is one of exclusion. Other conditions that can mimic ITP are acute infections, myelodysplasia, chronic DIC, drug-induced thrombocytopenia, and chronic liver disease with platelet sequestration.
- The distinction from congenital thrombocytopenia is especially important to avoid inappropriate treatment.

Treatment: Initial Management

- Patients who are incidentally discovered to have asymptomatic mild or moderate ITP can safely be followed with no treatment.
- Patients with platelet counts of more than 50×10^9/L usually do not have spontaneous, clinically important bleeding, and may undergo invasive procedures.

Emergency Treatment of Acute Bleeding Caused by Severe Thrombocytopenia

- Immediate platelet transfusion is indicated for patients with hemorrhagic emergencies. Despite having a presumably short platelet survival time, some patients have substantial posttransfusion increments in their platelet counts.

- IVIG may be given as a single infusion of 0.4 to 1.0 g/kg followed immediately by a platelet transfusion. IVIG, 1 g/kg per day for 2 days, increases the platelet count in most patients within 3 days.
- High doses of glucocorticoids, such as 1 g of methylprednisolone daily for 3 days, may cause a rapid increase in the platelet count.
- ε-Aminocaproic acid can be effective in controlling acute bleeding after failure of platelet transfusion and prednisone.

Glucocorticoids

- Glucocorticoid therapy likely decreases sequestration and destruction of antibody-sensitized platelets and may enhance platelet production.
- Prednisone, given in a dose of 1 mg/kg per day orally, is indicated for patients with symptomatic thrombocytopenia, and probably for all patients with platelet counts below 30 to 50×10^9/L who may be at increased risk for hemorrhagic complications.
- Sixty percent of patients will increase their platelet count to greater than 50×10^9/L, and approximately 25% will achieve a complete recovery. Most relapse when the prednisone dose is tapered or discontinued.
- The duration of prednisone therapy prior to consideration of splenectomy depends on the severity of the bleeding, the dose of prednisone required to maintain a response, and the risks of surgery.
- Long-term therapy with glucocorticoids can lead to many important side effects, including immunosuppression and osteoporosis.
- Courses of high-dose dexamethasone (40 mg/d for 4 days) is being used increasingly frequently in an attempt to induce a more sustained remission than the rather poor results obtained with standard prednisone therapy. Randomized clinical trials are necessary to prove that this therapy is superior to standard doses of prednisone, or whether the addition of other immunosuppressive agents (eg, rituximab) is of real value.

Intravenous Immunoglobulin

- IVIG is used in adults when a transient rise in platelet count is desired or when glucocorticoids are contraindicated.
- Initial dose is 2 g/kg given over 2 to 5 days. Comparable responses may occur with half this dose, or with 0.8 g/kg given once.
- Typical response is an increase in platelet count 2 or 3 days after the infusions begin, with return to pretreatment levels within several weeks.
- Fever, headache, nausea, and vomiting occur in approximately 25% of recipients, and aseptic meningitis occurs in 10%. Acute renal failure may occur, and hemolysis because of alloantibodies is also a side effect. Such doses of IVIG are a large volume load for patients with borderline cardiac function.

Anti-Rh(D) Immune Globulin

- Approximately 70% of patients receiving infusions of anti-Rh(D) antiserum at a dose of 50

μg/kg will respond with an increase in platelet count greater than 20×10^9/L, and half will have an increase greater than 50×10^9/L.
- In most patients, the response lasts longer than 3 weeks.
- Anti-Rh(D) is ineffective in Rh(D)-negative patients or following splenectomy.
- Side effects include alloimmune hemolysis, which is usually no more severe than that encountered with IVIG, but several deaths have been reported due to massive hemolysis. Anti-Rh(D) is less expensive than a standard course of IVIG.
- Headache, nausea, chills, and fever are much less frequent than with therapeutic doses of IVIG.

Splenectomy

- Sustained remission occurs in about two-thirds of patients who undergo splenectomy.
- The risks of operative bleeding complications with splenectomy are low even with severe thrombocytopenia, but it is prudent to have platelet preparations available in case of excessive intraoperative bleeding.
- Intravenous IVIG can induce a transient remission of thrombocytopenia and may be used to prepare for the operation.
- Most responses to splenectomy occur within several days. Responses after 10 days are unusual. The rapidity and extent of the response appear to correlate with durability of response.
- Splenectomy is associated with a small but significantly increased risk of severe infectious complications. All patients should be immunized with polyvalent pneumococcal, *Haemophilus influenzae* type b and meningococcal vaccines at least 2 weeks before surgery.
- One-half of patients who relapse after an initial response to splenectomy will do so within 6 months.

Removal of Accessory Spleens

- Accessory spleens are found at splenectomy in 15% to 20% of patients, and they may be present in as many as 10% of those refractory to splenectomy or who relapse after splenectomy.
- Remission after removal of an accessory spleen is unpredictable.

Thrombopoietin Receptor Agonists

- Two small molecule mimics of TPO have been approved by the US Food and Drug Administration for the treatment of refractory chronic ITP: romiplostim (N-plate), a “peptibody” composed of four copies of a TPO receptor binding peptide on an Ig scaffold, and eltrombopag (Promacta), a small organic molecule that is orally bioavailable. Several other thrombopoietin receptor agonists (TRAs) are currently undergoing clinical trials.
- Both drugs are potent stimulators of thrombopoiesis and rapidly (3–5 days) lead to major, dose-dependent increases (into the normal range) in platelet levels in the majority of patients.
- While on TRA therapy, hemorrhagic complications of thrombocytopenia occur less commonly and are less severe, and the use of coexistent ITP therapeutics and salvage agents is significantly reduced.

- Although studied carefully during clinical trials, with the exception of patients with liver cirrhosis, neither agent has been associated with a statistically significant increase in thrombotic complications, and in only a small number of patients have marrow reticulin fibrosis been noted. Eltrombopag has been associated with a low (approximately 4%) incidence of a modest rise in hepatic transaminases.
- Neither of the approved agents was initially thought to be disease modifying (ie, the platelet count remains normal only so long as the drug is used). However, recent publications indicate that a fraction of patients might have long-lasting remissions of ITP following a 6-month or longer course of a TRA. It should be noted that abrupt discontinuation of TRAs can lead to rebound thrombocytopenia more severe than the patient's baseline thrombocytopenia seen before institution of the drug.

Treatment: Chronic Refractory ITP

- Most other treatments available for patients with ITP who have relapsed after splenectomy have given inconsistent results and are often associated with significant risk. Refractory ITP presents an unusually complex clinical problem.
- Observation may be appropriate for asymptomatic patients, even those with platelet counts of less than 30×10^9/L.
- The goal of treatment is to achieve a platelet count that ensures hemostasis, not necessarily a normal platelet count.

Treatment of ITP During Pregnancy and Delivery

- It is extremely important to attempt to differentiate ITP from gestational thrombocytopenia.
- Early in pregnancy, treatment of maternal ITP should be the same as if the patient were not pregnant, using glucocorticoids in those patients whose symptoms require intervention.
- Splenectomy should be deferred if possible because ITP may improve after delivery. Therapy with IVIG may help delay splenectomy.
- In infants born to mothers with ITP, there is a 10% risk of a platelet count less than 50×10^9/L and a 4% risk of a platelet count less than 20×10^9/L.
- The severity of neonatal thrombocytopenia correlates with the severity of maternal thrombocytopenia. Treatment of the mother with glucocorticoids or with IVIG close to term has no effect on the platelet count of the infant.
- No satisfactory method is available to obtain accurate fetal platelet counts.
- Current practice is to recommend cesarean section only for obstetric indications.
- It is critical to monitor the newborn's platelet count during the first several days of life because severe thrombocytopenia may develop after delivery.

Childhood ITP

Clinical Features

- Peak incidence is from ages 2 to 4 years and is the same in both sexes until age 10 years, when female predominance appears.
- Bruises and petechiae are nearly universal presenting symptoms, usually of less than 1 to 2

weeks duration.
- Epistaxis, gingival bleeding, and gastrointestinal bleeding are uncommon.
- The frequency of a palpable spleen is the same as in unaffected children (approximately 10%).

Laboratory Features

- Most children present with platelet counts less than 20×10^9/L.
- Marrow examination is usually performed to rule out acute lymphocytic leukemia.

Course and Prognosis

- About 85% of patients selected for no specific treatment (eg, glucocorticoids or splenectomy) have a complete response within 6 months.
- Good prognostic features are abrupt onset, brief duration, and mild symptoms.
- Most responders develop no new purpura after the first week, and the platelet count is usually normal in 2 to 8 weeks.
- Purpura for more than 2 to 4 weeks before diagnosis is the most important predictor of chronic thrombocytopenia. Other factors are female sex, age greater than 10 years, and higher platelet count at presentation.
- Few children with ITP have critical complications, and even fewer die. Only 1% or less have intracerebral hemorrhage.

Treatment

- The need for treatment is controversial. No specific treatment is recommended by some for patients with bruising as the only symptom, regardless of the severity of the thrombocytopenia, but most patients receive treatment, more often with IVIG than with glucocorticoids.
- IVIG given 0.8 g/kg in a single dose or 2.0 g/kg in divided doses is expected to improve the platelet count significantly more rapidly than no treatment.
- Treatment has not been shown to decrease the risk of bleeding or death.
- Because of the risks of severe infection, splenectomy should be deferred for 6 to 12 months after diagnosis, and then recommended only for children with severe thrombocytopenia and significant bleeding symptoms.
- Splenectomy in children is associated with an increased risk of severe infection. In addition to all routine immunizations, polyvalent pneumococcal, *H influenzae* type b, and meningococcal vaccines should be given more than 2 weeks prior to splenectomy. Penicillin prophylaxis is routinely given to splenectomized children up to the age of 5 years.
- Efficacy of measures for therapy of chronic ITP in childhood is uncertain. Because the mortality is low and spontaneous remissions occur even after many years, potentially harmful treatments should be used only when there is a substantial risk of death or morbidity from hemorrhage.

CYCLIC THROMBOCYTOPENIA

- This rare disorder occurs predominantly in young women, usually related to the menstrual

cycle, but it also occurs in men and postmenopausal women. In some patients, there are parallel cycles of leukopenia.

- The pathogenesis may be autoimmune platelet destruction, increased platelet phagocytosis because of cyclic increments in macrophage colony-stimulating factor or cyclic decreases in platelet production.
- Although spontaneous remissions may occur, cyclic thrombocytopenia is chronic in most patients and may be a prodrome for marrow failure.
- Numerous therapies for cyclic thrombocytopenia have been attempted, with inconsistent success at best.

HEPARIN-INDUCED THROMBOCYTOPENIA (SEE ALSO CHAP. 90)

Etiology

- Heparin-induced thrombocytopenia (HIT) is an immune-mediated disorder caused by antibodies that recognize a neoepitope in platelet factor 4 exposed when it binds heparin. The result is activation of platelets, monocytes, and the coagulation cascade and, ultimately, thrombosis.

Clinical Features

- It should be noted that the platelet count of many, if not most patients drop by approximately 10% following the institution of heparin therapy. This may begin soon after heparin is started and may resolve even while heparin is continued. This form of thrombocytopenia is most frequent with full-dose therapy. It is not antibody-mediated and may be a result of platelet aggregation by heparin.
- Patients may present with a wide range of platelet counts, including levels close to or above normal, as long as the count has dropped by 50% from baseline.
- Unless recently exposed to heparin (< 100 days), the platelet count begins to fall 4 to 5 days after heparin therapy is instituted.
- The disease can occur with any heparin preparation: unfractionated heparin, low-molecular-weight heparins, and heparin-like compounds such as pentosan and danaparoid. All doses and routes of administration may also lead to HIT. Higher-molecular-weight fractions of heparin may interact more readily with platelets and thereby cause thrombocytopenia more frequently.
- HIT affects up to 5% of patients exposed to regular heparin and to lesser numbers of patients exposed to other forms of heparin.
- Thrombocytopenia may recur on readministration of heparin.
- Regardless of the degree of thrombocytopenia, the disease is the most hypercoagulable condition known.
- Venous thromboembolism is more commonly seen than is arterial thrombosis, although the latter is usually more striking. Thrombosis usually appears the first week after diagnosis and has high morbidity and mortality.

Laboratory Features

- Two assay prototypes for confirming the diagnosis are available. One measures the Ig

antibodies to the heparin/PF4 complex (antigen assay), and the other measures heparin-dependent antibodies that activate platelets (activation assay) in plasma or sera.

- Commercially available antigen assays measure either binding to PF4-heparin or PF4-polyvinylsulfate by enzyme-linked immunosorbent assay.
- Activation assays are not commercially established because specific platelet donors are needed each time, and donor platelets can vary greatly in their sensitivity to activation by HIT sera. One of the earliest and best-established activation assays, serotonin release assay, involves ^{14}C-serotonin release from platelets induced by HIT antibodies and heparin.
- In patients with a high clinical risk, heparin should be stopped and alternative treatment started even before laboratory results are available. A positive antigen test and particularly a progressive increase in the number of platelets over the following days are confirmatory.
- A negative antigen test does not rule out the diagnosis and should be repeated after 24 hours while the patient is undergoing alternative anticoagulant therapy. If the repeat assay is negative and platelet count does not increase, alternative diagnoses should be considered.

Prevention, Diagnosis, and Treatment

- The platelet counts of patients on heparin therapy should be obtained frequently.
- For patients requiring long-term anticoagulation, the best means of avoiding thrombocytopenia associated with thrombosis is to initiate therapy with a vitamin K antagonist or direct anticoagulant agent simultaneously with heparin so that therapeutic anticoagulation will be achieved before HIT is likely to occur.
- A clinical suspicion of HIT should be made if the platelet count falls below 100×10^9/L, or decreases by more than 50% from baseline and the decrease is unexplained by any other cause, or if a thromboembolic episode develops that is unexplained by other causes.
- Heparin therapy should be stopped once a strong clinical suspicion of HIT arises, and especially once a diagnosis is made.
- Several drugs available for anticoagulation in patients with HIT are now available, including argatroban, desirudin, bivalirudin, danaparoid, and fondiparinaux, which directly inhibit thrombin or fXa.
- Lepirudin prolongs the activated partial thromboplastin time (aPTT), so this test can be used to monitor effective dosing. Lepirudin induces antilepirudin antibodies in approximately half of patients who receive the drug. These antibodies rarely alter biologic activity but tend to prolong the drug's half-life, necessitating careful monitoring by aPTT. The availability of lepirudin is uncertain.
- Argatroban is synthesized from arginine and is rapidly metabolized in the liver. It affects both the aPTT and prothrombin time.
- Use of these direct thrombin or fXa inhibitors in HIT is efficacious; the incidence of thrombotic complication is reduced, perhaps by half, and the time to platelet count recovery is shortened. However, bleeding complications can occur.
- Lepirudin or argatroban should be given until patients recover from thrombocytopenia before adding and then switching to a prolonged course of an oral anticoagulant.

OTHER DRUG-INDUCED IMMUNOLOGIC THROMBOCYTOPENIAS

Etiology and Pathogenesis

- In these patients, thrombocytopenia is assumed to be a consequence of immune platelet destruction by drug-dependent antibodies. The target of antibody attack is usually composed of a drug-platelet surface protein complex.
- A vast number of drugs have been implicated as causing thrombocytopenia. Drugs for which modestly rigorous criteria for a causal effect are presented in Chap. 117, Table 117–7 of *Williams Hematology,* 9th ed.

Clinical and Laboratory Features

- Drug-induced thrombocytopenia typically produces profoundly low platelet counts.
- The time from initiating drug therapy to the development of thrombocytopenia averages 14 days, but may be as long as 3 years. With rechallenge, thrombocytopenia may appear within minutes, but almost always appears within 3 days.
- Patients may have nausea and vomiting, rash, fever, and abnormal liver function tests. Leukopenia may also develop.

Diagnosis

- A careful history is crucial. In addition to prescription medications, the patients should be asked about over-the-counter drugs, alternative therapies, soft drinks, mixers, and aperitifs that may contain quinine.
- Laboratory tests to detect drug-dependent antiplatelet antibodies remain largely investigational.
- The diagnosis can only be made by rechallenge with the drug after recovery from thrombocytopenia, but rechallenge can be dangerous because of the possibility of developing severe thrombocytopenia, even with very small doses of a drug.
- For patients who require therapy with multiple drugs, it may be appropriate to reintroduce each drug individually and to observe the patient for several days before adding another drug.

Treatment

- Withdrawal of the offending drug is essential.
- Prednisone therapy is commonly given but may not influence recovery.
- Major bleeding requires urgent intervention as for severe ITP: platelet transfusion, high-dose parenteral methylprednisolone, and possibly IVIG.

NEONATAL ALLOIMMUNE THROMBOCYTOPENIA

Etiology and Pathogenesis

- Pathogenesis is similar to neonatal alloimmune hemolytic disease except that fetal platelets rather than erythrocytes provide the antigenic challenge.
- Destruction of fetal platelets is caused by transplacentally acquired maternal antibodies directed against fetal-platelet–specific antigen inherited from the father.
- The platelet antigen HPA-1a, found in approximately 98% of the general population, provides

the most frequent immunogenic stimulus in persons of European ancestry. Other alloantigens are also implicated.

Clinical and Laboratory Features

- First-born children are often affected, indicating that fetal platelets cross the placenta during gestation. Recurrence with subsequent pregnancies is common.
- Because only 2% of the general population lacks the HPA-1a antigen, finding that the mother's platelets are HPA-1a-negative provides presumptive evidence of alloimmune origin. Neonatal alloimmune thrombocytopenia is in every respect more severe than thrombocytopenia in infants born to mothers with ITP, with death or neurologic impairment occurring in up to 25% of infants.
- Platelet counts usually recover in 1 to 2 weeks.

Prevention and Management

- Antenatal screening for neonatal alloimmune thrombocytopenia has been studied, but the cost-effectiveness of such a program has not been established.
- Management of neonatal alloimmune thrombocytopenia requires platelet transfusion, glucocorticoids, and IVIG.
- Maternal platelets are HPA-1a-negative and should be effective in transfusion. However, they require washing to remove maternal plasma containing antibodies and irradiation to prevent graft-versus-host disease.
- If HPA-1a-negative platelets are unavailable, random donor platelets plus IVIG treatment may be used.
- Management of subsequent pregnancies may require in utero sampling of fetal blood to obtain platelet counts and serial in utero platelet transfusions, procedures with significant risks for the fetus.
- Administration of IVIG and glucocorticoids to the mother may reduce the prevalence of in utero cerebral hemorrhage but is not effective in all patients.
- Delivery by scheduled cesarean section may reduce the risk of neonatal cerebral hemorrhage.

POST-TRANSFUSION PURPURA

- This acute, severe thrombocytopenia occurs 5 to 15 days after transfusion of a blood product and is associated with high-titer, platelet-specific alloantibodies.

Etiology and Pathogenesis

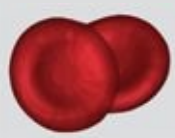
- Platelet destruction is caused by an alloantibody to a platelet-specific antigen.
- Anti-HPA-1a is present in more than 80% of cases, but alloimmunization to most other platelet-specific antigens has been reported.
- The mechanism of formation of the antibody is well established, but it remains uncertain how anti-HPA-1a antibodies can cause destruction of HPA-1a-negative platelets.

Clinical and Laboratory Features

- Most patients are women, and most are multiparous.
- Severe thrombocytopenia (platelet counts < 5×10^9/L) with major bleeding occurs several days after transfusion of 1 or more units of blood product, usually packed red cells.
- Fever often accompanies the inciting transfusion and the initial presentation.
- Antibodies to a platelet-specific alloantigen can be detected by appropriate serologic methods.
- Only after recovery can the patient's platelet types be determined.

Treatment, Course, and Prognosis

- Platelet transfusions are essential if there is severe, active bleeding, but these frequently lead to systemic reactions and the platelet count may not be increased.
- Glucocorticoids and IVIG are usually effective.
- Plasma exchange may be effective in 80% of patients.
- Thrombocytopenia begins to resolve after several days in most patients, but may be persistent and severe in some.



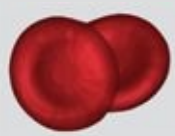
For a more detailed discussion, see Reyhan Diz-Küçükkaya and José A. López: Thrombocytopenia, Chap. 117 in *Williams Hematology*, 9th ed.

CHAPTER 74
Reactive (Secondary) Thrombocytosis

- The upper limit of a normal platelet count is usually between 350 × 10^9/L and 450 × 10^9/L depending on the clinical laboratory and specific method used.
- **Table 74–1** presents the major causes of elevation of the platelet count above the normal limit.
- Reactive thrombocytosis may persist for prolonged periods and resolve only with resolution of the underlying disorder.
- Thrombocytosis after recovery from thrombocytopenia ("rebound") usually peaks in 10 to 14 days.
- The platelet count after splenectomy may reach 1000 × 10^9/L or more within the first week and return to normal within about 2 months. Severe or persistent postsplenectomy thrombocytosis may be a result of persistent iron deficiency anemia or unmasking of primary thrombocythemia.
- There is no convincing evidence that therapy to reduce the platelet count or interfere with platelet function is of benefit in reactive thrombocytosis, with the possible exception of severe postsplenectomy thrombocytosis in patients with persistent hemolytic anemia, in which case aspirin therapy might be considered.

TABLE 74–1 MAJOR CAUSES OF THROMBOCYTOSIS

1. Clonal thrombocytosis
   a. Primary (essential) thrombocythemia
   b. Other myeloproliferative disorders (polycythemia vera, chronic myelogenous leukemia, primary myelofibrosis)
2. Familial thrombocytosis
3. Reactive (secondary) thrombocytosis
   a. Acute blood loss
   b. Iron deficiency
   c. Postsplenectomy, asplenic states
   d. Recovery from thrombocytopenia ("rebound")
   e. Malignancies
   f. Chronic inflammatory and infectious diseases (inflammatory bowel disease, connective tissue disorders, temporal arteritis, tuberculosis, chronic pneumonitis)
   g. Acute inflammatory and infectious diseases
   h. Response to exercise
   i. Response to drugs (vincristine, epinephrine, all-*trans*-retinoic acid, cytokines, and growth factors)
   j. Hemolytic anemia



For a more detailed discussion, see Kenneth Kaushansky: Reactive Thrombocytosis, Chap. 119 in *Williams Hematology,* 9th ed.

CHAPTER 75
Hereditary Platelet Disorders

Abnormalities of platelet function are expressed primarily by mucocutaneous bleeding. The most frequent laboratory abnormality is abnormal platelet aggregation or prolongation of the closure time in an automated platelet function analyzer. The clinical value of the bleeding time is questionable because of lack of reproducibility and poor correlation with clinical bleeding and should not be used.

- Hereditary qualitative platelet disorders classified according to the responsible abnormalities are presented in **Table 75–1**.

TABLE 75–1 INHERITED DISORDERS OF PLATELET FUNCTION

I. Abnormalities of glycoprotein adhesion receptors
- A. Integrin αIIbβ3 (glycoprotein IIb/IIIa; CD41/CD61): Glanzmann thrombasthenia
- B. Glycoproteins Ib (CD42b,c)/IX (CD42a)/V: Bernard-Soulier syndrome
- C. Glycoprotein Ibα (CD42b,c): Platelet-type (Pseudo-) von Willebrand disease
- D. Integrin α2β1 (glycoprotein Ia/IIa; VLA-2; CD49b/CD29)
- E. CD36 (glycoprotein IV)
- F. Glycoprotein VI

II. Abnormalities of platelet granules
- A. δ-Storage pool deficiency
- B. Gray platelet syndrome (α-storage pool deficiency)
- C. α,δ-Storage pool deficiency
- D. Quebec platelet disorder

III. Abnormalities of platelet signaling and secretion
- A. Defects in platelet agonist receptors or agonist-specific signal transduction (thromboxane A2 receptor defect, adenosine diphosphate [ADP] receptor defects [P2Y12, P2X1], epinephrine receptor defect, platelet-activating factor receptor defect)
- B. Defects in guanosine triphosphate (GTP)–binding proteins (Gαq deficiency, Gαs hyperfunction and genetic variation in extra-large Gαs, Gαi1 deficiency, CaLDAG-GEFI deficiency)
- C. Phospholipase C (PLC)-β2 deficiency and defects in PLC activation
- D. Defects in protein phosphorylation protein kinase C (PKC)-θ deficiency
- E. Defects in arachidonic acid metabolism and thromboxane production (phospholipase A2 deficiency cyclooxygenase [prostaglandin H2 sythase-1 deficiency], thromboxane synthase deficiency)

IV. Abnormalities of platelet coagulant activity (Scott syndrome)

V. Abnormalities of a cytoskeletal structural protein: β1 tubulin, filamin A

VI. Abnormalities in cytoskeletal linking proteins
- A. Wiskott-Aldrich syndrome protein (WASP)
- B. Kindlin-3: Leukocyte adhesion defect (LAD)-III; LAD-1 variant; integrin activation deficiency disease defect (IADD)

VII. Abnormalities of transcription factors leading to functional defects
- A. RUNX1 (familial platelet dysfunction with predisposition to acute myelogenous leukemia)
- B. GATA-1
- C. FLI1 (dimorphic dysmorphic platelets with giant α granules and thrombocytopenia; Paris-Trousseau/Jacobsen syndrome)
- D. GFI1B

Source: *Williams Hematology,* 9th ed, Chap. 120, Table 120–1.

ABNORMAL GLYCOPROTEIN (GP) IIB/IIIA (INTEGRIN AIIBB3, CD41/CD61): GLANZMANN THROMBASTHENIA

- Glanzmann thrombasthenia is characterized by severely reduced or absent platelet aggregation in response to many physiologic agonists because of abnormalities of platelet GP IIb and/or IIIa (see Table 75–1).

Etiology and Pathogenesis

- GPIIb/IIIa functions as receptor for fibrinogen and other adhesive glycoproteins.
- It is required for platelet aggregation induced by all agonists believed to function in vivo.
- Both GPIIb and GPIIIa are required for normal function, and defects in either component may cause thrombasthenia.
- Many different molecular biologic abnormalities have been described that affect expression or various functions of the two molecules.
- Inheritance of the disorder is autosomal recessive, but about 40% of patients are compound heterozygotes rather than homozygotes.

Clinical Features

- The most frequent bleeding symptoms in patients with Glanzmann thrombasthenia are menorrhagia, easy bruising, epistaxis, and gingival bleeding.
- Clinical expression does not correlate with the degree of abnormality of the laboratory findings, and the severity of bleeding symptoms can vary significantly during the life of an individual patient.
- Carriers are usually asymptomatic and have normal platelet function.

Laboratory Features

- Patients have normal platelet count and morphology.
- They have abnormal platelet aggregation to physiologic stimuli (eg, to ADP).
- Clot retraction is decreased or absent.
- There are many other abnormalities of platelet function of research interest.
- Autoantibodies to GPIIb/GPIIIa inhibits the function of normal platelets studied in plasma.

Differential Diagnosis

- Specific laboratory findings can distinguish other qualitative platelet disorders.
- von Willebrand disease, afibrinogenemia, hemophilia, and related disorders can be distinguished by specific laboratory tests.

Treatment

- Preventive measures include dental hygiene, avoidance of antiplatelet drugs, hepatitis vaccination early in life, and hormone therapy to avoid menorrhagia.
- Iron and folic acid therapy may be required in patients with continued bleeding.
- For management of bleeding, local therapy is given as appropriate, such as pressure dressings,

Gelfoam, and dental splints, and so on. Antifibrinolytic therapy may be helpful.
- Epistaxis may be particularly difficult to control.
- Platelet transfusions are given for serious hemorrhage, and packed red cell transfusions are often needed to correct blood loss anemia. All transfusions should be delivered through leukocyte-depletion filters.
- Antifibrinolytic agents (eg, ε-aminocaproic acid) are useful in patients with gingival bleeding or who are undergoing tooth extractions.
- Treatment of patients with Glanzmann thrombasthenia with recombinant factor VIIa (rFVIIa) has produced considerable, but not universal success, and rare thromboembolic complications have been reported in association with this therapy.
- With repeated platelet transfusion, alloimmunization occurs to platelet proteins such as human leukocyte antigen and GPIIb and/or GPIIIa.
- A few patients with severe bleeding have had allogeneic marrow hematopoietic stem cell transplantation, with success in some cases.

Prognosis
- Bleeding problems may be severe and frequent, but prognosis for survival is good.

GLYCOPROTEIN IB (CD42B, C), GP IX (CD42A), AND GP V: BERNARD-SOULIER SYNDROME

- Bernard-Soulier syndrome (BSS) is characterized by moderate thrombocytopenia, giant platelets, and failure of platelets to undergo selective von Willebrand factor (VWF) interactions as a result of abnormalities of the GP Ib/IX complex.
- The mechanisms leading to the thrombocytopenia and the giant platelets are not known.
- The abnormal platelet reactions with VWF and thrombin and the abnormalities of coagulant activity are related to the glycoprotein abnormalities.

Etiology and Pathogenesis
- Patients with BSS are deficient in GP Ib, GP IX, and GP V.
- Several qualitative abnormalities of GP Ib and GP IX have been identified. No defective forms of GP V have been identified.
- BSS is inherited as an autosomal recessive trait; an autosomal dominant form and acquired forms have also been reported.
- Six features contribute to the hemorrhagic diathesis: thrombocytopenia, abnormal platelet interactions with VWF, abnormal platelet interactions with thrombin, abnormal platelet coagulant activity, abnormal platelet interactions with P-selectin, and abnormal platelet interactions with leukocyte integrin αMβ2.

Clinical Features
- Epistaxis is the most common symptom. Ecchymoses, menometrorrhagia, gingival bleeding, and gastrointestinal bleeding also occur frequently.

- Symptoms vary considerably among patients, even those in a single family.

Laboratory Features

- Thrombocytopenia is found in nearly all patients, ranging from about 20×10^9/L to nearly normal levels.
- More than one-third of platelets are large; some are larger than lymphocytes.
- Platelets do not aggregate in response to ristocetin. In contrast to von Willebrand disease, this abnormality is not corrected by addition of normal plasma.
- Platelet coagulant activity may be reduced, normal, or increased.

Differential Diagnosis

- This is discussed in "Glanzmann Thrombasthenia."

Treatment and Prognosis

- These are similar to those for Glanzmann thrombasthenia.

ABNORMAL GP IB (CD42B, C): PLATELET-TYPE OR PSEUDO-VON WILLEBRAND DISEASE

- A heterogenous group of patients have mild to moderate bleeding symptoms, variable thrombocytopenia, variably enlarged platelets, and diminished plasma levels of high-molecular-weight multimers of VWF.

Etiology and Pathogenesis

- GP Ib/IX is the receptor for VWF.
- Abnormal forms of GP Ib cause enhanced binding of VWF, leading to reduction in high-molecular-weight multimers in plasma, and perhaps reduction in platelet survival time.
- Specific mutations have been demonstrated in some patients.
- The condition is inherited as an autosomal dominant trait.

Clinical Features

- Patients have mild to moderate mucocutaneous bleeding.

Laboratory Features

- Some patients have thrombocytopenia and large platelets.
- Plasma VWF concentration is reduced, especially the high-molecular-weight multimers.
- Enhanced platelet aggregation in response to low concentrations of ristocetin is not corrected by normal plasma. (In type II von Willebrand disease, this abnormality is corrected by normal plasma.)

Treatment

- Administration of VWF or desmopressin (DDAVP) to increase endogenous release of VWF may be beneficial in low doses but can cause thrombocytopenia because of increased binding to platelets.
- Patients should be instructed to avoid aspirin or other antiplatelet agents.
- Platelet transfusion may be beneficial if thrombocytopenia is severe.

OTHER GLYCOPROTEIN DEFICIENCIES

- A mild bleeding disorder has been described in association with decreased platelet content of GP Ia and GP IIa.
- Deficiency of GP IV occurs in a small number of people who have no bleeding disorder.
- Deficiency of GP VI has been found in patients with mild bleeding disorders.

WISKOTT-ALDRICH SYNDROME

- Wiskott-Aldrich syndrome is characterized by small platelets, thrombocytopenia, recurrent infections and eczema, although only a minority of patients have all features of the disorder.

Etiology and Pathogenesis

- Wiskott-Aldrich syndrome is inherited as an X-linked trait. In fact, if the eczema and immunodeficiency are minimal, the condition is termed X-linked thrombocytopenia. Female carriers of Wiskott-Aldrich syndrome have normal platelet counts and normal platelet size as they select against mutant X-chromosome *WAS* gene.
- Mutations of a Wiskott-Aldrich syndrome protein (WASP) occur in many, but not all, patients with the Wiskott-Aldrich syndrome and X-linked thrombocytopenia.
- WASP is a cytoplasmic protein, expressed in all hematopoietic stem cell–derived lineages. It plays a major role in organization and regulation of the actin cytoskeleton.
- A defect has also been found in sialophorin (CD43), a glycoprotein found on lymphocytes, monocytes, neutrophils, and platelets, but its role in pathogenesis is not clear.
- Deficiencies in GP Ia, Ib, IIb/IIIa, and IV have been found in some, but not all, patients.
- Deficiency of the platelet storage pool of adenine nucleotides and abnormal platelet energy metabolism are found in some patients.
- The thrombocytopenia is believed to be a result of diminished platelet survival, but ineffective thrombopoiesis may also play a role.
- The cause of the small platelets is unknown.

Clinical Features

- Mucocutaneous bleeding
- Recurrent infections
- Eczema
- Increased risk of development of lymphoma, even in childhood
- Possibly autoimmune diseases, including hemolytic anemia and thrombocytopenia

Laboratory Features

- Thrombocytopenia, often with counts of 20 × 10^9/L or less, and with reduced platelet volume, may occur.
- Platelet aggregation and release of dense body contents are variably abnormal.
- Defects in both humoral and cellular immunity, especially deficiency in immune response to polysaccharide antigens.

Treatment

- Patients should be specifically instructed to avoid aspirin or other antiplatelet agents.
- Splenectomy improves thrombocytopenia and may lead to increased platelet size and improved function.
- Allogeneic hematopoietic stem cell transplantation may be curative.

PLATELET GRANULE DEFICIENCY STATES

δ-Storage Pool Deficiency

- This is a usually mild bleeding disorder with abnormalities in the second wave of platelet aggregation and deficiencies in the contents of the dense granules of platelets.
- There is predisposition to hematologic malignancies in some families.
- It occurs as a primary disorder or in association with inherited multisystem disorders:
  — Hermansky-Pudlak syndrome
  — Chédiak-Higashi syndrome
  — Wiskott-Aldrich syndrome (see above discussion)
  — Others (less frequently)
- The mode of inheritance of the primary disorder is not well defined, but autosomal dominance has been reported. The forms associated with other disorders are inherited following the pattern of the primary disease.

Clinical Features

- Severe bleeding may occur in patients with Hermansky-Pudlak syndrome; in others bleeding is mild to moderate.
- Mucocutaneous bleeding, excessive bruising, and epistaxis are common.
- Excess bleeding after surgery or trauma also may occur.

Laboratory Features

- The results of platelet function tests vary from patient to patient and may vary in the same patient over time.
- Variable abnormalities of second wave of platelet aggregation are characteristic.

Differential Diagnosis

- See “Glanzmann Thrombasthenia,” above.

Treatment

- Avoid antiplatelet drugs.
- The bleeding associated with surgery may be decreased by therapy with glucocorticoids.
- Platelet transfusion may be helpful if bleeding is severe.

Hermansky-Pudlak Syndrome

- Hermansky-Pudlak syndrome is unusually common in patients from northwest Puerto Rico, affecting 1 in 1800 individuals.
- This syndrome causes variable oculocutaneous albinism and the absence of dense platelets.
- Linkage analysis of patients from areas where Hermansky-Pudlak syndrome is relatively common led to the identification of the abnormal gene in these patients, then termed *HPS1*.
- The *HPS1* gene encodes a 700–amino acid protein that, along with HPS4, comprises BLOC-3 component of the granule exocytosis machinery.

Gray Platelet Syndrome (α-Granule Deficiency)

- α-Granule membranes form abnormal vesicular structures rather than granules.
- Platelets are deficient in α-granule contents, including fibrinogen and VWF.
- α-Granule deficiency (gray platelet) may be diagnosed by measuring platelet factor-4 and/or β-thromboglobulin in platelets.

Clinical Features

- Mild hemorrhagic manifestations are usual, but severe bleeding has been reported.

Laboratory Features

- Platelets on blood films are pale, gray, ghost-like, oval, and larger than normal.
- Thrombocytopenia is common, and the platelet count may be below 50×10^9/L.
- Platelet aggregation is often normal or nearly so, but may be abnormal.

Differential Diagnosis

- See “Glanzmann Thrombasthenia,” above.
- Degranulated platelets may also be seen in myelodysplastic and myeloproliferative disorders.

Treatment

- General measures should be used as in Glanzmann thrombasthenia.
- DDAVP or antifibrinolytic therapy may be beneficial.
- Platelet transfusion is indicated for serious hemorrhage.
- Thrombocytopenia may be improved by glucocorticoid therapy.

α, δ-Storage Pool Deficiency

- There are moderate to severe defects in both α and δ granules.
- Clinical and laboratory features are similar to δ-storage pool deficiency.

Quebec Platelet Disorder

- The early description of this autosomal dominant disorder included severe bleeding after trauma, mild thrombocytopenia, decreased functional platelet factor V, and normal plasma factor V.
- Epinephrine-induced platelet aggregation is normal.
- Subsequent studies demonstrated that the platelets of these patients had markedly reduced levels of multimerin and thrombospondin, and both reduced levels and proteolysis of a number of α-granule proteins, including factor V, fibrinogen, VWF, fibronectin, and osteonectin.
- The defect in these patients' platelets appears to be excessive plasmin generation as a result of increased expression of urokinase-type plasminogen activator (uPA); increased megakaryocyte expression of the uPA gene due to an abnormality in a *cis* regulatory element may be the primary abnormality.

ABNORMALITIES OF PLATELET COAGULANT ACTIVITY (SCOTT SYNDROME)

- Patients whose platelets fail to facilitate thrombin generation are defined as having defects in platelet coagulant activity. Only a few patients with isolated defects in platelet coagulant activity have been described.
- There is decreased translocation of platelet phosphatidyl serine to the outer membrane leaflet, which results in decreased binding of factors Va-Xa and VIIIa-IXa and hence, a diminished rate of blood clotting.

Clinical Features

- Bleeding, sometimes severe, occurs after trauma, dental extractions, delivery, or surgery. Epistaxis and menorrhagia also occur.
- Bleeding is not primarily mucocutaneous, in contrast to other qualitative platelet disorders.

Laboratory Features

- *Serum* prothrombin time is abnormal.
- Assays for "platelet factor 3" are abnormal.

Differential Diagnosis

- The abnormal serum prothrombin time distinguish patients with abnormalities of platelet coagulant activity.

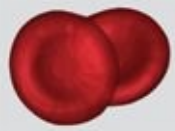
Treatment

- Platelet transfusions have been effective for prevention and treatment.
- Prothrombin complex concentrates may be effective but may induce thrombosis.

ABNORMALITIES OF PLATELET AGONIST RECEPTORS, SIGNAL

TRANSDUCTION, AND SECRETION

- A number of defects in the complex process of platelet activation that cause usually mild hemostatic disorders with rare episodes of severe clinical expression have been described.
- The most common pattern is blunted platelet aggregation with absence of the second wave of aggregation on exposure to ADP, epinephrine, or collagen, and decreased release of dense granule contents. Such patients have been lumped together, more out of convenience than because of an understanding of the mechanism, under the rubric of primary secretion defects, activation defects, or signal transduction defects.
- Occasional patients demonstrate defects in the thromboxane receptor, one of the ADP receptors ($P2Y_{12}$, $P2Y_1$ and $P2X_1$), the epinephrine receptor or the GTP-binding proteins that mediate signaling for these heptahelical G-protein coupled receptors, or the signaling intermediates that mediate these platelet activation pathways, such as cyclooxygenase, thromboxane synthase, phospholipase (PL) Cβ or PLCθ.



For a more detailed discussion, see A. Koneti Rao and Barry S. Coller: Hereditary Qualitative Platelet Disorders, Chap. 120 in *Williams Hematology*, 9th ed.

CHAPTER 76
Acquired Platelet Disorders

- The clinical manifestations of bleeding disorders are usually mild but may be severe if there is an accompanying hemostatic abnormality or a local lesion that may be predisposed to bleed.
- The usual laboratory abnormalities focus on abnormal platelet aggregation tests, but these results do not necessarily predict the risk of clinical bleeding.
- **Table 76–1** lists the principal types and causes of acquired qualitative platelet abnormalities.

TABLE 76–1 ACQUIRED QUALITATIVE PLATELET DISORDERS

Drugs that affect platelet function
Aspirin and other nonsteroidal anti-inflammatory drugs
P2Y12 antagonists (clopidogrel, prasugrel, ticagrelor)
PAR1 thrombin receptor antagonist (vorapaxar)
Integrin αIIbβ3 receptor antagonists (abciximab, eptifibatide, tirofiban)
Drugs that increase platelet cyclic adenosine monophosphate
Antibiotics
Anticoagulants and fibrinolytic agents
Cardiovascular drugs
Volume expanders
Psychotropic agents and anesthetics
Oncologic drugs
Foods and food additives
Hematologic disorders associated with abnormal platelet function
Chronic myeloproliferative neoplasms
Leukemias and myelodysplastic syndromes
Dysproteinemias
Acquired von Willebrand syndrome
Systemic disorders associated with abnormal platelet function
Uremia
Antiplatelet antibodies
Cardiopulmonary bypass
Liver disease
Disseminated intravascular coagulation
Infection with HIV

Source: *Williams Hematology*, 9th ed, Chap. 121, Table 121–1.

DRUGS THAT AFFECT PLATELET FUNCTION

- **Table 76–2** lists drugs known to interfere with platelet function. Drugs are the most common cause of abnormal platelet function.
- Some drugs can prolong platelet function tests and cause or exacerbate a bleeding disorder.
- Some drugs induce abnormal platelet function tests but do not cause bleeding.

TABLE 76–2 DRUGS THAT AFFECT PLATELET FUNCTION

Nonsteroidal anti-inflammatory drugs Aspirin, ibuprofen, sulindac, naproxen, meclofenamic acid, mefenamic acid, diflunisal, piroxicam, tolmetin, zomepirac, sulfinpyrazone, indomethacin, phenylbutazone, celecoxib
P2Y12 antagonists Clopidogrel, prasugrel, ticagrelor
PAR1 receptor antagonist Vorapaxar
Integrin αIIbβ3 antagonists Abciximab, eptifibatide, tirofiban
Drugs that affect platelet cyclic adenosine monophosphate levels or function Prostacyclin, iloprost, dipyridamole, cilostazol
Antibiotics Penicillins Penicillin G, carbenicillin, ticarcillin, methicillin, ampicillin, piperacillin, azlocillin mezlocillin, sulbenicillin, temocillin Cephalosporins Cephalothin, moxalactam, cefoxitin, cefotaxime, cefazolin Nitrofurantoin Miconazole
Anticoagulants, fibrinolytic agents, and antifibrinolytic agents Heparin Streptokinase, tissue plasminogen activator, urokinase ε-Aminocaproic acid
Cardiovascular drugs Nitroglycerin, isosorbide dinitrate, propranolol, nitroprusside, nifedipine, verapamil, diltiazem, quinidine
Volume expanders Dextran, hydroxyethyl starch
Psychotropic drugs and anesthetics Psychotropic drugs Imipramine, amitriptyline, nortriptyline, chlorpromazine, promethazine, fluphenazine, trifluoperazine, haloperidol Anesthetics Local: dibucaine, tetracaine, Cyclaine, butacaine, nupercaine, procaine, cocaine General: halothane
Oncologic drugs Mithramycin, daunorubicin, BCNU, ibrutinib
Miscellaneous drugs Ketanserin
Antihistamines Diphenhydramine, chlorpheniramine, mepyramine
Radiographic contrast agent Iopamidol, iothalamate, ioxaglate, meglumine diatrizoate, sodium diatrizoate
Foods and food additives ω-3 Fatty acids, ethanol, Chinese black tree fungus, onion extract ajoene, cumin, turmeric

Source: *Williams Hematology*, 9th ed, Chap. 121, Table 121–2.

Aspirin

- Two isoforms of cyclooxygenase have been identified (COX-1 and COX-2). COX-1 is constitutively expressed by many tissues, including platelets, the gastric mucosa, and endothelial cells. COX-2 is undetectable in most tissues, but its synthesis is rapidly induced in

cells such as endothelial cells, fibroblasts, and monocytes by growth factors, cytokines, endotoxin, and hormones.

- Aspirin irreversibly inhibits both COX-1 and COX-2 and thereby interferes with normal platelet function, such as aggregation with ADP or epinephrine.
- Platelet function testing is markedly prolonged by aspirin in patients with coagulopathies or platelet abnormalities.
- The platelet function abnormalities remain prolonged for up to 4 to 7 days after aspirin is discontinued.
- Patients taking aspirin may have increased bruising, epistaxis, and gastric erosions that may bleed.
- A meta-analysis of clinical trials indicates that aspirin doses varying from 50 to 1500 mg daily are equally efficacious in preventing adverse cardiovascular and cerebrovascular events. This has led many to suggest that the lowest effective doses should be prescribed to minimize gastrointestinal toxicity. Nonetheless, even low doses of aspirin can be associated with gastrointestinal hemorrhage.

Other Nonsteroidal Anti-inflammatory Drugs

- Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit COX reversibly and usually for less than 4 hours.
- Because ibuprofen, and probably other NSAIDs, binds to COX and blocks its acetylation by aspirin, coadministration of NSAIDs may impair the irreversible effects of aspirin on platelets. For this reason, patients who require both medications should ingest aspirin at least 2 hours before the ingestion of other NSAIDs.
- The effect of piroxicam may last for days because of the long half-life of the drug.

COX-2 Inhibitors (Coxibs)

- The coxibs, designed to be relatively more specific for COX-2 versus COX-1, were intended to reduce pain and inflammation with fewer gastric side effects than traditional NSAIDs. However, clinical trials revealed that administration of coxibs was associated with cardiovascular toxicity (myocardial infarction, stroke, edema, exacerbation of hypertension), due at least in part to inhibition of prostaglandin I_2 (PGI_2) synthesis. On the basis of these results, rofecoxib and valdecoxib were withdrawn from the market (valdecoxib was also associated with cases of Stevens-Johnson syndrome) and a Black Box Warning regarding serious cardiovascular events was added to prescribing information for celecoxib, the only coxib now available in the United States.
- Clinical evidence indicates there is no excess cardiovascular risk from daily doses of celecoxib of 200 mg or less. Because traditional NSAIDs also inhibit COX-2 and several clinical trials have indicated excess cardiovascular events with the use of some of these agents, a warning has also been added to their prescribing information.

Antibiotics

- Most penicillins cause dose-dependent prolongation of platelet function tests, probably by binding to the platelet membrane and thereby interfering with platelet function. For example,

platelet aggregation is frequently abnormal.

- Inhibition of platelet function is maximal after 1 to 3 days of therapy and persists for several days after treatment is discontinued.
- Clinically significant bleeding occurs much less frequently than do abnormal platelet functions.
- Patients with coexisting hemostatic defects are particularly vulnerable.
- Some cephalosporins cause problems similar to those caused by the penicillins.

Thienopyridines

- These drugs (ticlopidine and clopidogrel) are used as antithrombotic agents in arterial disease. They are more effective than aspirin or other NSAIDs for secondary prevention of cardiovascular events.
- Both thienopyridines are prodrugs that depend on metabolites to competitively inhibit the platelet P2Y12 ADP receptor. Effects of ticlopidine and clopidogrel on platelet aggregation may be seen within 24 to 48 hours of the first dose but are not maximal for 4 to 6 days.
- At therapeutic doses, they inhibit platelet function as much or more than aspirin, and the effects appear to be additive to those of aspirin.
- Ticlopidine administration has been associated with potentially serious hematologic complications, including neutropenia ($<1.2 \times 10^9$/L in 2.4% of individuals), and less commonly, aplastic anemia and thrombocytopenia. In addition, at least 1 in 5000 patients treated with ticlopidine develop thrombotic thrombocytopenic purpura.
- Results from a large clinical trial suggest that hematologic complications may be less common with clopidogrel.
- A loading dose of 300 mg of clopidogrel followed by a daily dose of 75 mg per day shortens the time required for the maximal antiplatelet effect. The presence of the common polymorphism of cytochrome P450, termed CYP2C19, results in lower levels of the active metabolite in patients. This effect can lead to decreased inhibition of platelet function, and elevated risk for major adverse cardiovascular events.
- It appears that the added benefit of double antiplatelet therapy (eg, addition of aspirin) for most patients is small, and at times dangerous.

GP IIb/IIIa Receptor Antagonists

- Abciximab, eptifibatide, and tirofiban are three GP IIb/IIIa inhibitors approved by the US Food and Drug Administration that are structurally dissimilar but all rapidly impair platelet aggregation. Abciximab is a human-murine chimeric Fab fragment, eptifibatide is a cyclic heptapeptide, and tirofiban is a nonpeptide mimetic.
- Inhibitors of GP IIb/IIIa interfere with platelet function and are used as antithrombotic agents in patients with coronary atherosclerosis, usually in combination with heparin. These drugs predispose to bleeding, which is more severe with higher doses of heparin.
- Platelet transfusions appear to reverse the platelet functional defect in patients receiving abciximab, the Fab fragment of a monoclonal antibody. The effectiveness of platelet transfusion in patients receiving low-molecular-weight antagonists (tirofiban, eptifibatide) is not established.

- Thrombocytopenia has occurred within 24 hours of initiating therapy with all types of GP IIb/IIIa antagonists as a result of preformed antibodies to a ligand induced epitope on the platelet GP IIb/IIIa. Platelet counts less than 50×10^9/L have been reported in approximately 1% to 4% of patients.
- In most cases of profound thrombocytopenia, a platelet count obtained 2 to 4 hours after initiating therapy provides evidence of a significant decrease in platelet count, although cases of delayed thrombocytopenia have been observed after abciximab. Thrombocytopenia usually reverses when drug administration is stopped.

Anticoagulants, Fibrinolytic Agents, and Antifibrinolytic Agents

- Heparin inhibits platelet function under some circumstances, but predisposes to bleeding primarily because of its anticoagulant effect.
- Platelet function may be altered during fibrinolytic therapy, but this appears not to be primarily responsible for hemorrhagic complications.

Volume Expanders

- Dextran interferes with platelet function by adsorption to the platelet surface but does not predispose to bleeding, unless administered with low-dose heparin.
- Hydroxyethyl starch may predispose to bleeding, especially at doses exceeding 20 mL/kg of a 6% solution, and large volumes of gelatin-based volume expanders may affect platelet function.

FOODS

- Diets rich in fish oils containing omega-3 fatty acids may interfere with platelet function and prolong the bleeding time.

ABNORMAL PLATELET FUNCTION IN UREMIA

Etiology and Pathogenesis

- Patients may have a modest bleeding diathesis because of defects in platelet adhesion, aggregation, or procoagulant activity because of poorly understood mechanisms. For example, uremic plasma can inhibit the adhesion of normal platelets to deendothelialized human umbilical artery segments, whereas uremic platelets adhere normally in the presence of normal plasma, but for unknown reasons. Moreover, increased nitric oxide synthesis by endothelial cells or platelets is at least partially responsible for defective platelet function in uremia.
- Anemia appears to be a major contributor to the adhesion defect and the prolonged bleeding times of uremic patients because of vascular rheology; normal red cell numbers force platelets to the endothelial surface of a column of flowing blood. Correction of the hematocrit to approximately 30% normalizes this defect.
- Concurrent medications (eg, aspirin, heparin) may add to the abnormalities.
- Thrombocytopenia may contribute to the bleeding tendency. If the platelet count is less than

100×10^9/L, causes of thrombocytopenia other than uremia must be considered.

Clinical and Laboratory Features

- The hemostatic defect in uremia is usually mild.
- The most common bleeding sites are skin, gastrointestinal, and genitourinary tracts.
- Patients with gastrointestinal bleeding frequently have a predisposing anatomic lesion.
- Serious bleeding requiring surgical intervention after biopsy is uncommon, and usually is a result of factors other than uremia.
- If bleeding occurs, a search for the cause should be initiated without assuming uremia is responsible.
- The bleeding time is often prolonged but does not quantitate risk of hemorrhage.

Treatment

- Intensive dialysis can correct the abnormal bleeding in many patients.
- Desmopressin (DDAVP) given intravenously or subcutaneously improves platelet function in most uremic patients. For patients who need repeated doses, intranasal administration can be attempted.
- DDAVP is usually given at a dose of 0.3 µg/kg intravenously over 15 to 30 minutes (maximum dose 20 µg).
- Repeated administration at intervals of 12 to 24 hours has been reported, but tachyphylaxis may occur.
- Transfusion of red cells to achieve a hematocrit of greater than or equal to 30% may improve pathological bleeding. Combined transfusion of red cells and DDAVP may offer added benefit.
- Conjugated estrogens improve platelet function in most patients with uremia. The dose is usually 0.6 mg/kg intravenously for 5 days.
- Cryoprecipitate may diminish bleeding, but the results are uncertain and risks significant.

ANTIPLATELET ANTIBODIES

Etiology and Pathogenesis

- Nearly all cases occur in association with immune thrombocytopenia.
- Antiplatelet antibodies may interfere with platelet function by binding to functional membrane components; others may activate platelets and induce aggregation and secretion.

Clinical Features

- Platelet dysfunction should be considered if a patient with Immune thrombocytopenia (ITP) or systemic lupus erythematosus develops mucocutaneous bleeding with a platelet count above the usual bleeding level.

Laboratory Features

- Platelet aggregation abnormalities are found in most patients. The most frequent pattern is absence of aggregation response to a low dose of collagen, and absence of the second wave in

response to ADP or epinephrine.

Treatment

- Treatment is directed to the underlying immune thrombocytopenia.

CARDIOPULMONARY BYPASS

Etiology and Pathogenesis

- Thrombocytopenia is a consistent feature of bypass surgery. Typically, platelet counts decrease to 50% of presurgical levels by 25 minutes after the initiation of bypass, but thrombocytopenia can occur within 5 minutes and may persist for as long as several days.
- Approximately 5% of patients experience excessive postoperative bleeding after extracorporeal bypass. Roughly half of the bleeding is due to surgical causes, and much of the remainder is due to qualitative platelet defects and hyperfibrinolysis.
- The platelet defect is probably caused by activation and fragmentation.
- Drugs such as heparin and protamine may interfere with platelet function.

Clinical Features

- Platelet dysfunction is a possible cause of excessive postoperative bleeding.

Laboratory Features

- Platelet aggregation to several agonists is abnormal.
- The platelet count is typically reduced by 50% during bypass and may remain low for several days.

Treatment

- Surgical causes of bleeding, incomplete neutralization of heparin, and persistence of hypothermia must be considered.
- Patients with prolonged bleeding time and excessive postoperative blood loss may respond to DDAVP.
- Aprotinin has been demonstrated to be beneficial, possibly through an antifibrinolytic effect.
- Transfusion with appropriate blood components may be necessary.

CHRONIC MYELOPROLIFERATIVE NEOPLASMS

- A bleeding diathesis occurs in approximately 20% of patients with these diseases, and thrombophilia in approximately 40%.
- Multiple functional platelet abnormalities have been demonstrated.

Clinical Features

- Bleeding or thrombosis occurs in about one-third of patients and is a common cause of morbidity and mortality in these patients.

- Bleeding usually involves the skin or mucous membranes but may occur after surgery or trauma.

Laboratory Features

- None of the platelet function abnormalities is unique to a particular myeloproliferative neoplasm, and none is predictive of bleeding or thrombosis.
- Thrombocytosis is common, but the degree is not predictive of bleeding or thrombosis unless greater than 1000×10^9/L, where an acquired von Willebrand disease can cause bleeding due to platelet adsorption of von Willebrand factor.

Treatment

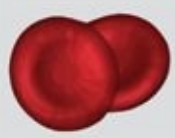
- Therapy should be reserved for symptomatic patients or those about to undergo surgery.
- Treatment should be directed to the underlying disorder.
- DDAVP may benefit storage pool defects or acquired von Willebrand disease in these patients.
- Aspirin may be helpful in patients with thrombosis but predisposes to bleeding.

ACUTE LEUKEMIA AND MYELODYSPLASTIC SYNDROMES

- Thrombocytopenia is the most common cause of bleeding, but platelet dysfunction may also contribute.
- Platelets may be morphologically abnormal, aggregate abnormally, and have decreased procoagulant activity.
- Bleeding usually responds to platelet transfusion and treatment of underlying disease.

PARAPROTEINEMIAS

- Platelet dysfunction occurs commonly due to direct interaction of the monoclonal protein with the platelets.
- Treatment is to reduce plasma levels of abnormal immunoglobulins by cytoreductive therapy or plasmapheresis.



For a more detailed discussion, see Charles S. Abrams, Sanford J. Shattil, and Joel S. Bennett: Acquired Qualitative Platelet Disorders, Chap. 121 in *Williams Hematology,* 9th ed.

CHAPTER 77

The Vascular Purpuras

DEFINITIONS

- *Purpura* is the extravasation of red cells from vasculature into the skin and/or subcutaneous tissues.
- *Petechiae* are red-purple lesions less than 2 mm in diameter.
- *Purpura* describes red-purple lesions 2 mm to 1 cm.
- *Ecchymoses* are red-purple lesions greater than 1 cm.
- *Erythema* is reddened skin due to increased capillary flow.
- *Telangiectasia* is dilated superficial capillaries.
- Erythema and telangiectasia blanch with pressure, and petechiae and purpura do not. This can be easily demonstrable with a glass microscope slide.

PATHOPHYSIOLOGY

- Hemostatic mechanisms may be unable to protect against minor vascular trauma.
- Vessels and surrounding tissues may be weakened structurally.
- Transmural pressure gradient may be too great.
- Palpability may result from:
  — Extravascular fibrin deposition
  — Cellular infiltration due to inflammation or malignancy

NONPALPABLE PURPURA

Increased Transmural Pressure Gradient

- Increased intrathoracic pressure caused by coughing, vomiting, weight lifting, etc. may cause petechiae of the face, neck, and upper thorax.
- Venous valvular incompetence or tight clothing may cause petechiae on the lower extremities.

Decreased Mechanical Integrity of the Microvasculature or Supporting Tissues

- Actinic (senile) purpura is red to purple irregular patches on the extensor surfaces of the forearm and hands.
- Glucocorticoid excess causes bright red purpuric lesions in thin, fragile skin on flexor and extensor surfaces of both arms and legs.
- Vitamin C deficiency (scurvy) results in a susceptibility of lysyl and prolyl hydroxylases to inactivation, which are two key enzymes in collagen biosynthesis in the skin. This leads to

follicular hyperkeratosis, petechiae, and perifollicular purpura with entrapped corkscrew hairs. Large ecchymoses and hemorrhagic gingivitis, stomatitis, and conjunctivitis may occur.

- Ehlers-Danlos syndrome is characterized by easy bruising in types IV and V, but this may occur with other types as well.
- Pseudoxanthoma elasticum may be associated with recurrent mucosal hemorrhages.
- In amyloidosis, infiltration of blood vessel walls may lead to increased vascular fragility and petechiae or purpura.
- The female easy-bruising syndrome (purpura simplex) is purpura or ecchymoses occurring predominantly in women, frequently on the thighs. This may be related to hormonal changes and can be aggravated by nonsteroidal anti-inflammatory drug (NSAID) ingestion.

TRAUMA

- Physical trauma can cause cutaneous bleeding. The history, shape, and location of the lesions may suggest the etiology.
- Factitial purpura usually presents as medium to large ecchymoses on the lower extremities of patients who appear unconcerned about the lesions.

SUNBURN

- Acute sunburn may be sufficiently severe to have a petechial component.

INFECTIONS

- Purpura may occur with bacterial, fungal, viral, or rickettsial infections, or with parasitic infestations, including protozoan, often as a consequence of a complex, multifactorial process. Special forms include:
  - — Bacterial sepsis due to various organisms can cause petechiae or purpura, macules or papules, hemorrhagic bullae, erosions, ulcers, or widespread ecchymoses and cutaneous infarctions (see “Purpura Fulminans,” below).
  - — Ecthyma gangrenosum may accompany infections with *Pseudomonas* sp, *Klebsiella* sp, *Aeromona hydrophilia*, or *Escherichia coli* in patients with severe granulocytopenia or immune compromise. Lesions begin as erythematous or purpuric macules and progress to hemorrhagic or necrotic vesicles or bullae, then to edematous, hemorrhagic plaques, and finally to indurated painless ulcers.
  - — Meningococcemia may cause erythematous papules that progress to widespread petechiae, purpura, and ecchymoses. Acrocyanosis and peripheral gangrene may occur.
  - — Scarlet fever is characterized by a diffuse, erythematous rash often with confluent petechiae in skin folds (Pastia lines). Streptococcal pharyngitis without scarlet fever may also be associated with petechiae.
  - — Rickettsial infections cause cutaneous lesions, beginning as urticarial macules and progressing to petechiae, ecchymoses, hemorrhagic bullae, and extensive skin necrosis.
  - — In Lyme disease, the characteristic cutaneous lesion is erythema migrans, an annular,

expanding plaque that may contain a purpuric macule or papule, or a hemorrhagic bulla.

EMBOLIC PURPURA

- Cholesterol crystals that embolize from atheromata in the aorta and in the lower extremities may produce petechiae and purpura, livedo reticularis, nodules, ulcers, or cyanosis and gangrene along with eosinophilia in the blood. Typically, this occurs in patients with atherosclerotic burden that undergo vascular procedures.
- Fat emboli may occur after severe trauma or after liposuction and cause petechiae of the upper extremities, thorax, and/or conjunctivae. The clinical picture may also include shock and respiratory insufficiency.

HYPERCALCEMIA

- Chronic hypercalcemia may lead to hemorrhagic cutaneous necrosis because of subcutaneous and vascular calcifications.

NEOPLASIA

- Petechiae or purpura may occur because of infiltration of the skin with neoplastic cells from a variety of malignancies, including leukemias, myeloma, or macroglobulinemia.

PIGMENTED PURPURIC ERUPTIONS

- Schamberg and Majocchi diseases are characterized by petechiae and purpura on a background of red-brown or orange hyperpigmentation, usually on the lower extremities.
- Similar lesions may be produced by cutaneous T-cell lymphoma, drug or chemical hypersensitivity, allergic or irritant contact dermatitis, and hyperglobulinemic purpura.

PYODERMA GANGRENOSUM

- Affected patients present with a nodule, pustule, or hemorrhagic bulla that rapidly becomes an ulcer with an erythematous base and violaceous or blue margin surrounded by erythema.
- This condition is associated with a number of diseases, including inflammatory bowel disease, rheumatoid arthritis, and hematologic malignancies.

INTRA-ABDOMINAL HEMORRHAGE

- Purpura or ecchymoses may develop around the umbilicus (Cullen sign) or in the flanks (Grey-Turner sign) in patients with intra-abdominal hemorrhage (eg, acute pancreatitis).

COUMARIN NECROSIS

- Coumarin necrosis occurs in about 1 in 500 patients receiving coumarin drugs.
- The onset is sudden after 2 to 14 days of drug therapy, with painful erythematous patches that progress to hemorrhagic and necrotic plaques, nodules, and bullae.
- Women are more commonly affected, and lesions most often involve thighs, buttocks, or breasts.
- Coumarin necrosis is more likely to occur in patients with protein C deficiency and patients with heparin-induced thrombocytopenia.

PURPURA FULMINANS

- Affected patients may present with widespread ecchymoses, often involving the extremities, abdomen, or buttocks.
- This condition is often seen in association with infection and/or disseminated intravascular coagulation, but it may be idiopathic or occur in infants with homozygous protein C or protein S deficiency.

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

- This condition may be associated with erythematous cutaneous lesions with central necrosis, hemorrhagic bullae, petechiae, purpura, or ecchymoses, likely related to microvascular thrombosis.

ANTIPHOSPHOLIPID SYNDROME

- Patients with this disorder may develop a variety of cutaneous manifestations, including ecchymoses, subungual splinter hemorrhages, and extensive cutaneous necrosis, all likely related to microvascular thrombosis (see Chap. 84).

DRUG REACTIONS

- Reactions to any of a large number of drugs may lead to petechiae or purpura in the absence of thrombocytopenia.

AUTOERYTHROCYTE SENSITIZATION

- This disorder is characterized by painful ecchymoses appearing without explanatory trauma.
- The cause is not established, but in some patients hypersensitivity to some component of the erythrocyte membrane may be responsible.
- Many patients have underlying psychiatric disorders, and lesions have been factitial in some.

PALPABLE PURPURA

Henoch-Schönlein Purpura (Small-Vessel Vasculitis)

- This syndrome is a leukocytoclastic vasculitis of unknown cause involving precapillary, capillary, and postcapillary vessels.
- Lesions may be palpable purpura, urticarial papules, plaques, or hemorrhagic vesicles or bullae which can progress to larger, stellate, reticulate, and necrotic lesions.
- Lesions are usually symmetric on legs and buttocks, and are often associated with fever.
- It is predominantly a disease of children between ages 2 and 20 years. Several environmental triggers precede onset, such as viral (upper respiratory infections, hepatitis B virus, hepatitis C virus, parvovirus B19, and HIV) and bacterial (*Streptococcus* species, *Staphylococcus aureus*, and *Salmonella* species) infections in children. Adult disease is precipitated by medications (NSAIDs, angiotensin-converting enzyme inhibitors, and antibiotics), food allergies, vaccinations, and insect bites.
- Arthralgias and abdominal pain usually accompany the rash, and melena and signs of peritoneal irritation are common.
- Proteinuria and hematuria occur in 40%. In older children and adults, renal disease may be progressive in 10% to 20%.
- IgA1 immunoglobulins and complement components may be deposited in involved cutaneous and renal vessels.
- Therapy is usually initiated with glucocorticoids, but the success rate is low. Ultimate prognosis is almost uniformly good.

Sweet Syndrome

- Also referred to as acute, febrile neutrophilic dermatosis, Sweet syndrome is characterized by the acute manifestation of painful erythematous and violaceous papules, nodules, and plaques accompanied by fever and elevated neutrophil count.
- These papules, which most commonly appear on face, neck, and upper extremities, present a central yellowish discoloration and tend to coalesce forming well-circumscribed, irregularly bordered plaques. Classically more prominent in middle age women, this syndrome is associated with a complex cytokine dysregulation. Other manifestations include respiratory and urinary infections, and autoimmune disorders.

Behçet Disease (Variable-Vessel Vasculitis)

- Besides its classification as a neutrophilic dermatosis, Behçet disease is also an inflammatory disorder that affects multiple organ systems.
- Clinical features are chronic and relapsing cutaneous manifestations, such as palpable purpura, infiltrative erythema, and papulopustular lesions, as well as oral mucosal and genital ulcers, arthralgias, and gastrointestinal and central nervous system involvement.

Serum Sickness

- Serum sickness refers to the systemic manifestation of immune complex formation and deposition.

- Serum sickness associated with infection or medical therapy can result in characteristic lesions.
- The use of antithymocyte globulin for marrow failure results in 75% of patients developing serpiginous bands of erythema and purpura on the sides of their hands and feet.
- Cutaneous lesions such as urticarial and morbilliform eruptions predominate, although palpable purpura and erythema multiforme are also often seen.

Erythema Multiforme

- Erythema multiforme (EM) is a cutaneous disorder characterized by the development of crops of well-demarcated, erythematous target lesions with central clearing, most commonly representing a hypersensitivity reaction triggered by infection or drug exposure.
- The severity of this disorder ranges from mild (EM minor), to severe, (EM major or Stevens-Johnson syndrome). EM has been reported to be triggered by a number of viruses (most common herpes simplex, but also adenovirus, cytomegalovirus, and HIV), and medications (sulfonamides, penicillins, bupropion, phenylbutazone, phenytoin, NSAIDs, adalimumab).
- A cellular allergic reaction coupled with impaired histamine metabolism due to decrease in histamine-N-methyltransferase activity may be causative. Treatment for mild cases is supportive, while glucocorticoid use is often warranted in severe cases.

Churg-Strauss Syndrome

- Churg-Strauss syndrome is characterized by granulomatous inflammation in the lungs associated with asthma and eosinophilia.
- Cutaneous findings such as ulcers, papules, palpable purpura, cutaneous nodules, and infarcts of fingers and toes are encountered in 50% to 80% of cases.
- It can be limited to the skin. Eosinophilia accompanies elevated IgE levels and a positive P-antineutrophil cytoplasmic antibody (ANCA). Granulomatous inflammation and necrotizing vasculitis of small to medium sized blood vessels are present histologically.

Acute Hemorrhagic Edema of Infancy

- This disorder is composed of a triad of fever; iris-like or medallion-like large purpuric, painful cutaneous lesions; and edema appearing in children age 4 months to 2 years.
- The onset is sudden, with spontaneous recovery in 1 to 3 weeks.
- The cutaneous lesions are limited to cheeks, eyelids, ears, and extremities and genitalia.
- Pathology is leukocytoclastic vasculitis with vascular deposits of immunoglobulins and complement components.

Vasculitis Associated with Other Diseases

- Palpable purpura may occur in several other disorders characterized by vasculitis:
  — Collagen vascular diseases
  — Systemic vasculitides, including polyarteritis nodosa (medium vessel vasculitis) or Wegener granulomatosis (granulomatous vasculitis)
  — Hypersensitivity vasculitis, associated with drug reactions or infections or idiopathic
  — Paraneoplastic, in association with any of a variety of neoplasms, including the

hematologic malignancies
— Long-distance walkers, who may develop purpuric vasculitis lesions on the legs

Cryoglobulinemia

- Cryoglobulinemia may involve a single component, IgA, IgG, or IgM, occurring in essential monoclonal gammopathy, macroglobulinemia, myeloma, or lymphoma.
- Cold-insoluble complexes of IgG with IgM have anti-IgG activity, or similar complexes containing other immunoglobulin components, may occur in association with a variety of diseases.
- Skin lesions occur with both types of cryoglobulin, including macular or palpable purpura, acral hemorrhagic necrosis, livedo reticularis, or hemorrhagic bullae.

Hyperglobulinemic Purpura of Waldenström

- This condition usually occurs in women between ages 18 and 40 years, often in association with another disease.
- Crops of petechiae appear on the lower legs and ankles, recurring at intervals of days to months.
- Patients have polyclonal hypergammaglobulinemia due to elevated levels of IgA, IgG, and IgM.

Cryofibrinogenemia

- Cold-insoluble fibrinogen may be found as a primary disorder or secondary to neoplastic, thromboembolic, or infectious disorders, usually with laboratory evidence of disseminated intravascular coagulation.
- Cutaneous manifestations are similar to those described for cryoglobulinemia, above.

Primary Cutaneous Diseases

- Primary cutaneous diseases, including allergic contact dermatitis, drug eruptions, acne vulgaris, insect bites, and dermatitis herpetiformis, may present with purpuric papules and vesicles that look like septic or vasculitis lesions.

DISORDERS SIMULATING PURPURA

Telangiectasias

- Hereditary hemorrhagic telangiectasia (Rendu-Osler-Weber):
  — Inheritance is autosomal dominant. More than 80% of all cases of HHT are caused by mutations in either *ENG* (endoglin, *HHT1*) or *ALK1* (*ACVRL1*, *HHT2*).
  — The disease is characterized by widespread dermal, mucosal, and visceral telangiectasias.
  — Recurrent epistaxis is the most common problem, but bleeding may occur from any site.
  — Arteriovenous fistulae may occur, especially in the lungs, and may require surgical resection.
  — Treatment involves local therapy to accessible lesions. Hormonal therapy may be used for

epistaxis or gastrointestinal bleeding. Lysine analogs (tranexamic acid or ε-aminocaproic acid) have been beneficial in some cases.

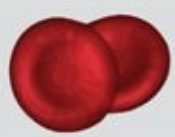
- Spider angiomata are telangiectasias that occur in chronic liver disease, a limited form of scleroderma (CREST syndrome: calcinosis, Raynaud syndrome, esophageal dysmotility, sclerodactyly, telangiectasia), and AIDS have a prominent central feeding vessel, which is easily occluded, leading to blanching of the lesion.
  — They may be confused with lesions of hereditary hemorrhagic telangiectasia.

Kaposi Sarcoma

- Kaposi lesions may mimic petechiae, purpura, or ecchymoses on either skin or mucosae.

Extramedullary Hematopoiesis

- Cutaneous sites of extramedullary hematopoiesis appear as dark red, blue, or blue-gray macules in infants with congenital viral infections, hemolysis associated with Rh incompatibility, hereditary spherocytosis or twin transfusion syndrome, and in adults with primary myelofibrosis.



For more detailed information, see Doru T. Alexandrescu and Marcel Levi: The Vascular Purpuras, Chap. 122 in *Williams Hematology*, 9th ed.

PART X

DISORDERS OF COAGULATION PROTEINS

CHAPTER 78

Hemophilia A and B

GENERAL ASPECTS

- Hemophilia A and hemophilia B are caused by inherited deficiencies of factor VIII and factor IX, respectively.
- Both result from decreased production of the deficient factor, production of a factor with decreased functional activity, or a combination of these two abnormalities.
- The activated form of factor IX, factor IXa, is a serine protease that functions to activate factor X.
- Activated factor VIII, factor VIIIa, serves as a cofactor, forming a complex with factor IXa on the platelet surface, that dramatically accelerates the rate of factor X activation by factor IXa.
- In patients with hemophilia, clot formation is delayed because thrombin generation is markedly decreased. The clot that does form is hemostatically ineffective, leading to excessive bleeding.
- Because deficiency of either factor VIII or factor IX causes an inability to activate factor X, the clinical characteristics and approach to treatment of hemophilia A and hemophilia B are similar.
- Both hemophilia A and B are X-linked recessive disorders, affecting only males, with rare exceptions (**Figure 78–1**). Approximately 30% of mutations arise de novo.
- Hemophilia is found worldwide in all ethnic groups. Hemophilia A is estimated to occur in 1 of 10,000 male births and hemophilia B in 1 of 25,000 to 30,000 male births.

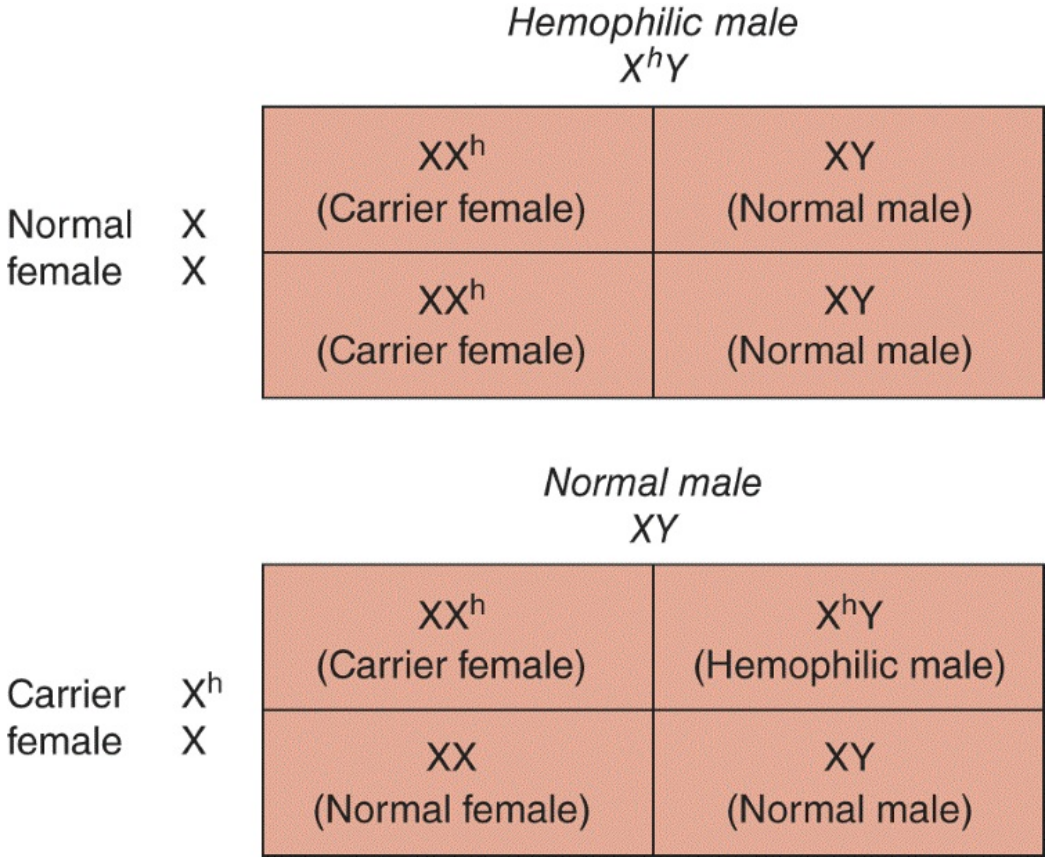


FIGURE 78–1 Inheritance pattern of hemophilia A. X is normal; X^h has an abnormal X chromosome with the hemophilic gene; Y is normal; XX is a normal female; XY is a normal male; XX^h is a carrier female; X^hY is a hemophilic male. (Source: *Williams Hematology*, 9th ed, Chap. 123, Fig. 123–1.)

HEMOPHILIA A

Clinical Features

- **Table 78–1** shows a clinical classification of hemophilia A based on factor VIII levels.
- Hemostasis is generally normal with levels in excess of 30%.
- The factor VIII level remains constant throughout the patient's life, and it is similar in other affected members of the kindred but varies between kindreds.
- Hemarthrosis accounts for 75% of bleeding episodes in patients with severe hemophilia A.
- The most frequent sites are the knees, followed by the elbows, ankles, shoulders, wrists, and hips.
- The acute form of hemarthrosis is characterized by initial mild pain without physical findings, followed by more intense pain, swelling and warmth of the joint, and decreased range of motion.
- The patient may have mild fever. Significant or sustained fever suggests infection in the joint.
- When bleeding stops, the blood resorbs and symptoms subside over several days.
- Repeated bleeding into the joint results in synovial hypertrophy and inflammation, with limitation of motion and a tendency for more frequent bleeding in that joint (target joint).
- Eventually, repeated hemorrhage into the joints causes destruction of the articular cartilage, synovial hyperplasia, and joint deformity with muscle atrophy and soft tissue contractures (**Figure 78–2**).

- Hematomas may develop after bleeding into muscles or subcutaneous tissues (**Figure 78–3**).
- Intramuscular hematomas occur most often in thigh, buttocks, calf muscles, and forearm.
- Hematomas may stabilize and slowly resorb without treatment, but in individuals with moderate or severe hemophilia, they often enlarge progressively and dissect in all directions. This can cause compression of adjacent organs, nerves, or blood vessels, sometimes leading to permanent sequelae. Hematomas may obstruct the airway.
- Pseudotumors are large, organized, encapsulated hematomas that may slowly expand and compress surrounding structures.
- Central nervous system hemorrhage, the most common cause of bleeding mortality, occurs spontaneously or after trauma. The onset of symptoms is usually prompt but may be delayed by several days.
- Virtually all patients with severe hemophilia have episodes of hematuria, which may cause renal colic because of clots in the ureters, but is seldom life-threatening.
- Postsurgical bleeding, often delayed by hours to several days, is associated with poor wound healing.
- Extraction of permanent teeth in patients with hemophilia may be followed by prolonged bleeding. Life-threatening pharyngeal or sublingual hematomas may follow extractions or regional block anesthesia.
- Inhibitory antibodies to factor VIII may develop in patients receiving replacement therapy (discussed below).

TABLE 78–1 CLINICAL CLASSIFICATION OF HEMOPHILIA A AND B

Classification	Hemophilia A Factor VIII Level	Hemophilia B Factor IX Level	Clinical Features
Severe	≤ 1% of normal (≤ 0.01 U/mL)	≤ 1% of normal. (≤ 0.01 U/mL)	1. Spontaneous hemorrhage from early infancy. 2. Frequent spontaneous hemarthroses and other hemorrhages requiring clotting factor replacement.
Moderate	1%–5% of normal (0.01–0.05 U/mL)	1%–5% of normal (0.01–0.05 U/mL)	1. Hemorrhage secondary to trauma or surgery. 2. Occasional spontaneous hemarthroses.
Mild	6%–30% of normal (0.06–0.30 U/mL)	6%–40% of normal (0.06–0.40 U/mL)	1. Hemorrhage secondary to trauma or surgery. 2. Rare spontaneous hemorrhage.

Source: *Williams Hematology*, 9th ed, Chap. 123, Table 123–1.

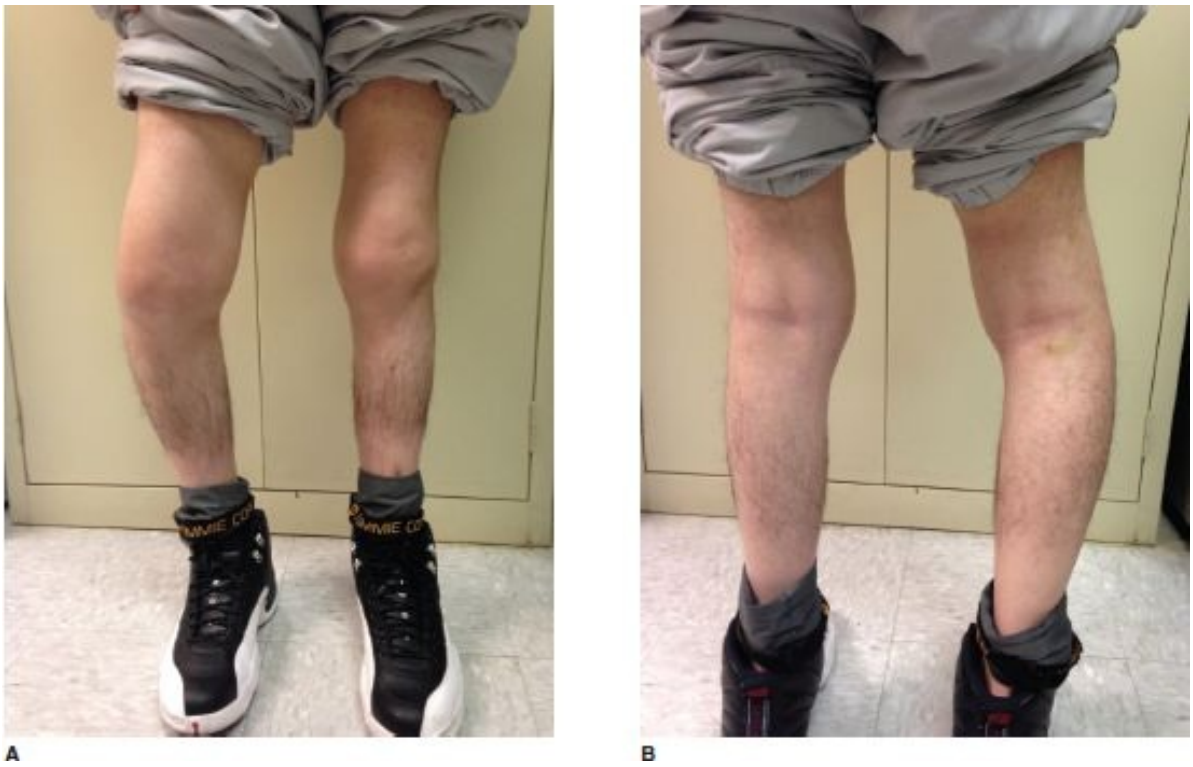


FIGURE 78–2 Hemophilic arthropathy. The chronic effects of repeated hemorrhage into the knee of a severely affected hemophilic patient are seen. Note swelling and deformity with atrophy of muscle tissue. (Source: *Williams Hematology*, 9th ed, Chap. 123, Fig. 123–4.)

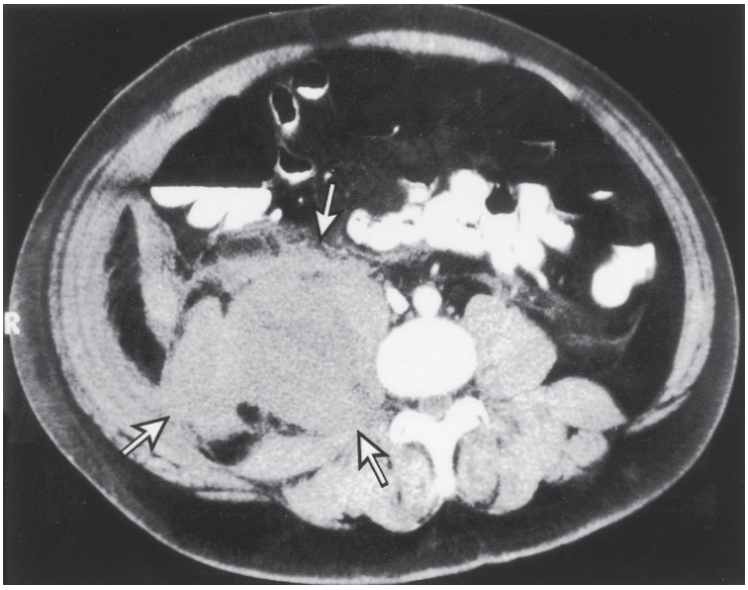


FIGURE 78–3 Computed tomography scan of a retroperitoneal hematoma in a patient with severe hemophilia A. Extent of the hematoma is indicated by the *arrows*. (Source: *Williams Hematology*, 9th ed, Chap. 123, Fig. 123–8.)

Laboratory Findings

- Hemophilia A causes prolongation of the activated partial thromboplastin time (aPTT), which is corrected by the addition of an equal volume of normal plasma. The prothrombin time is normal.
- A specific assay for factor VIII activity is required for definitive diagnosis.
- Immunologic assays coupled with clotting assays permit detection of dysfunctional factor VIII molecules.

Carrier Detection and Prenatal Diagnosis

- The average factor VIII level of carrier females is 50%, but occasional carriers have levels less than 30% and may have excessive bleeding with trauma or surgery.
- The family history is important for carrier detection (see **Figure 78–1**).
- Molecular genetics techniques are available to identify carriers.
- Prenatal diagnosis can be made from fetal cells obtained by amniocentesis or by chorionic villus biopsy.

Differential Diagnosis

- Hemophilia A must be distinguished from hemophilia B and from other congenital disorders of coagulation that prolong the aPTT, such as factor XI and XII deficiencies.
- Hemophilia A must be distinguished from von Willebrand disease (especially the Normandy variant), an acquired inhibitor of factor VIII, and combined congenital deficiency of factor VIII and factor V.

Treatment

General

- Avoid aspirin, other antiplatelet agents, and intramuscular injections.
- Treat bleeding episodes promptly.
- Consider prophylaxis in severely affected patients.
- Home treatment should be available to all patients.
- Plan surgical procedures carefully.
- Hemophilia should preferably be treated in a designated Hemophilia Treatment Center.

Desmopressin

- Deamino-8-D-arginine vasopressin (DDAVP) is often useful in the treatment of mild to moderate hemophilia A and symptomatic carrier females. Administration of 0.3 µg/kg intravenously can increase factor VIII levels of most of these patients two- to three-fold.
- The peak effect is in 30 to 60 minutes.
- Adverse reactions include flushing, rarely hyponatremia (mostly in children, can be prevented by water restriction), and angina in patients with coronary disease.
- Tachyphylaxis occurs with repeated doses.
- An intranasal preparation is also available.

Replacement with Factor Concentrates

- Bleeding episodes in patients with hemophilia A can be managed by replacing factor VIII (**Table 78–2**).
- Commercial concentrates prepared from human plasma have been treated to inactivate viruses, including HIV and hepatitis B and C viruses. Hepatitis A and parvovirus are not inactivated by the treatment, and infection with these viruses has been transmitted.
- Recombinant factor VIII concentrates appear to be both safe and effective. These may be formulated with human plasma albumin, and are more expensive.
- The various available concentrates appear to differ little in safety, efficacy, or convenience.

TABLE 78–2 CURRENTLY AVAILABLE FACTOR VIII PRODUCTS[a]

	Origin	Viral Inactivation
Intermediate purity		
Humate P[b]	Plasma	Pasteurization[c]
High purity		
Koate DVI[b]	Plasma	Solvent-detergent,[d] heat treated[i]
Alphanate[b]	Plasma	Solvent-detergent, heat treated[i]
Ultrapure[e]		
Hemofil M	Plasma	Solvent-detergent[d]
Monoclate P	Plasma	Pasteurization[c]
Recombinant		
Advate[h]	CHO cells[f]	Solvent-detergent[d]
Recombinate[e]		
Kogenate FS[e]	BHK cells[g]	Solvent-detergent
Helixate FS[e]	BHK cells[g]	Solvent-detergent
Xyntha[h]	CHO cells[f]	Solvent-detergent, nanofiltration
Eloctate[h]	HEK cells[j]	Solvent-detergent

[a]Additional concentrates are available in Europe.
[b]Contains VWF.
[c]Pasteurization at 60°C (140°F) for 10 h.
[d]Solvent-detergent: tri-n-butyl phosphate (TNBP) + polysorbate 80.
[e]Human albumin added; insignificant VWF.
[f]Chinese hamster ovarian cells.
[g]Baby hamster kidney cells.
[h]Not exposed to human or animal protein during manufacture.
[i]Heat treated at 80°C (176°F) for 72 h.
[j]Human embryonic kidney cells.
Source: *Williams Hematology*, 9th ed, Chap. 123, Table 123–2.

Factor VIII Dosage (Table 78–3)

TABLE 78–3 DOSES OF FACTOR VIII FOR TREATMENT OF HEMORRHAGE*

Site of Hemorrhage	Desired Factor VIII Level (% of normal)	Factor VIII Dose† (U/kg body weight)	Frequency of Dose‡ (every no. of hours)	Duration (days)
Hemarthroses	30–50	~25	12–24	1–2
Superficial intramuscular hematoma	30–50	~25	12–24	1–2
Gastrointestinal tract	50-100	50	12	7–10
Epistaxis	30–50	~25	12	Until resolved

Oral mucosa	30–50	~25	12	Until resolved
Hematuria	30–100	~25–50	12	Until resolved
Central nervous system	50–100	50	12	At least 7–10 days
Retropharyngeal	50–100	50	12	At least 7–10 days
Retroperitoneal	50–100	50	12	At least 7–10 days

*Mild or moderately affected patients may respond to 1-deamino-8-D-arginine vasopressin (DDAVP), which should be used in lieu of blood or blood products whenever possible.

†Factor VIII may be administered in a continuous infusion if the patient is hospitalized. After initial bolus, approximately 150 U of factor VIII per hour usually are sufficient in an average-size adult. Doses are given every 12 to 24 hours.

‡The frequency of dosing and duration of therapy can be adjusted, depending on the severity and duration of the patient's bleeding episode.

Source: *Williams Hematology*, 9th ed, Chap. 123, Table 123–3.

- The dose of factor VIII can be determined by multiplying the patient's weight in kilograms by half the needed percent correction of the factor level. For example, for a 70-kg patient with a less than 1% factor VIII level who needs a 100% correction, the dose would be 70 (kg) × 100%/2 = 3500 U. The full contents of mixed factor vials should be infused.
- The half-life of factor VIII is 8 to 12 hours. Factor levels may be maintained between 50% and 100% by giving half the loading dose every 8 to 12 hours.
- Reconstituted factor VIII concentrates may be administered by continuous intravenous infusion. After an initial loading dose to raise factor VIII to the desired level, 150 to 200 U per hour are administered.

Antifibrinolytic Agents

- Antifibrinolytic agents are useful adjunctive therapy for mucosal bleeding, and particularly so for dental extractions, but are contraindicated if the patient has hematuria.
- Tranexamic acid is given at an oral dose of 0.5 to 1 g four times daily
- ε-Aminocaproic acid (EACA) can be given orally in a loading dose of 4 to 5 g followed by 1 g/h, or 4 g every 4 to 6 hours for 2 to 8 days, depending on the severity of the bleeding episodes. Another regimen of EACA is 4 g every 4 to 6 hours orally for 2 to 8 days, depending on the severity of the bleeding episode.
- Fibrin glue, a mixture of fibrinogen and factor XIII applied locally to a bleeding site and then clotted with thrombin, may be useful adjunctive therapy for bleeding from circumcision, dental, or orthopedic procedures, including removal of large pseudotumors.

Treatment of Specific Types of Bleeding Episodes (see Table 78–2)

- Superficial cuts and abrasions are managed with local pressure.
- Epistaxis may require replacement of factor VIII to levels of 50% of normal.
- Hematuria is often mild and needs no replacement therapy but may persist and require replacement to levels greater than 50%, with replacement continuing until the bleeding stops. Patients should be advised to drink a lot of fluids, to avoid obstructing clots in the urinary tract.
- Prior to endoscopy, factor VIII should be replaced to at least the 50% level. A single infusion may suffice, but if the procedure is complicated by bleeding, replacement must be continued until the bleeding stops.

- For expanding soft-tissue hematomas, replacement therapy should be started immediately and continued until the hematoma begins to resolve.
- Hemarthroses should be treated promptly to minimize degenerative changes, deformity, and muscle wasting. For chronic bleeding into a "target" joint, replacement to 100% for 6 to 8 weeks may be indicated.
- Retropharyngeal and retroperitoneal hematomas and any central nervous system bleeding require replacement of factor VIII to normal (100%), or nearly normal, levels for 7 to 10 days.
- Major surgical procedures require factor VIII replacement to normal levels before operation and maintenance of normal levels for 7 to 10 days, or until healing is well underway.
- The patient's factor VIII levels should be measured during surgery and once or twice daily postoperatively and the dose of factor VIII adjusted accordingly.
- Home therapy has facilitated prompt treatment of hematomas and hemarthroses and markedly improved the morbidity and mortality of the disease.
- Severely afflicted patients who receive prophylactic therapy with 50 units of factor VIII/kg body weight three times a week have markedly decreased frequency of arthropathy and other long-term complications of hemophilia.
- Transplantation of a normal liver can result in cure of hemophilia, but this has been done rarely.
- Gene therapy for hemophilia is being extensively investigated.

Course and Prognosis

- Unless treated properly, patients develop complications of recurrent bleeding, as noted under "Clinical Features," above.
- The introduction of replacement therapy with factor VIII concentrates in the 1960s led to a significant reduction in the morbidity and mortality from bleeding in hemophilia but introduced serious complications such as infection with HIV, liver disease from hepatitis B and C, and the development of antifactor VIII antibodies.
- Since 1985, factor VIII concentrates have been treated to destroy HIV and hepatitis viruses, with virtual elimination of infection with these agents. However, HIV infection and chronic liver disease from hepatitis B and C are still prevalent in older patients with hemophilia.

Factor VIII Inhibitors in Patients with Hemophilia A

- Factor VIII inhibitors are antibodies, most often IgG, usually IgG4 subclass, that interfere with the interaction of factor VIII with its cofactors and activators.
- Factor VIII inhibitors react slowly, and inactivation of factor VIII requires incubation with the inhibitor for 1 to 2 hours at 37°C.
- Laboratory diagnosis of a factor VIII inhibitor requires that an appropriate dilution of the patient's plasma when mixed with normal plasma will neutralize only factor VIII and no other factor that influences the aPTT (factors IX, X, XI, XII, prekallikrein, or high-molecular-weight kininogen).
- Patients with factor VIII inhibitors are classified as "high" responders if their baseline inhibitor levels are above 10 Bethesda units (BU) or if their inhibitor level rises above 10 BU after receiving factor VIII replacement. "Low" responders have factor VIII inhibitor levels

below 10 BU even after receiving factor VIII replacement.

- High-responder patients with major bleeding and initial inhibitors below 10 BU can be treated with high doses of human or porcine factor VIII concentrates in efforts to neutralize the inhibitor and still provide enough factor VIII for hemostasis.
- High-responder patients with initial inhibitor levels greater than 10 BU usually will not respond to any doses of human factor VIII, nor to porcine factor VIII if the inhibitor is cross-reactive.
- High-responders should be treated with recombinant factor VIIa or another factor VIII inhibitor-bypassing agent for minor bleeding episodes or for major bleeding if the inhibitor level is high or if factor VIII replacement is ineffective (**Table 78–4**).
- Low-responders can be treated with recombinant factor VIIa or another factor VIII inhibitor-bypassing agent for major or minor bleeding. In addition, they can be treated with high-dose human or porcine factor VIII for major bleeding (see **Table 78–4**).
- Recombinant factor VIIa is the preferred factor VIII inhibitor-bypassing agent. Factor VIIa is believed to activate factor X on the surfaces of activated platelets, and factor Xa can then interact with factor Va and convert prothrombin to thrombin. The effects of factor VIIa may be localized because activated platelets are found principally at sites of injury. Prothrombin complex preparations are probably of benefit also because of similar effects of activated coagulation factors in these products.
- In some patients, administration of daily doses of factor VIII can reduce the inhibitor titer to undetectable levels, and such immune tolerance regimens offer a promising approach to eradication of factor VIII inhibitors. Bleeding episodes that occur during induction of tolerance can be treated with inhibitor-bypassing agents.
- Details of treatment of patients with inhibitors are presented in Chap. 123 of *Williams Hematology*, 9th ed.

TABLE 78–4 TREATMENT OF INHIBITORS IN HEMOPHILIA A PATIENTS

Type of Patient	Initial Titer	Minor Hemorrhage*	Major Hemorrhage*
High responder	< 5 BU	Recombinant factor VIIa; FEIBA	Factor VIII;† recombinant factor VIIa; FEIBA
High responder	> 5 BU	Recombinant factor VIIa; FEIBA	Recombinant factor VIIa; FEIBA; plasma exchange
Low responder	< 5 BU	Recombinant factor VIIa; FEIBA	High-dose factor VIII; recombinant factor VIIa; FEIBA

BU, Bethesda unit; FEIBA, factor VIII inhibitor bypassing activity.

*Choices of agents for treatment of major and minor hemorrhage are listed. Some physicians will choose the first product listed as the agent of choice, but the choice varies among physicians.

†High dose of factor VIII may overcome an initial low-titer inhibitor, although an anamnestic response can be expected in high responders.

Source: *Williams Hematology*, 9th ed, Chap. 123, Table 123–5.

Spontaneous Factor VIII Inhibitors

- Autoantibodies against factor VIII may appear in patients without hemophilia. This occurs idiopathically in older adults, in pregnant and postpartum women, and in patients with immunologic disorders (eg, systemic lupus erythematosus and rheumatoid arthritis).

- Clinical manifestations include spontaneous ecchymoses and intramuscular hemorrhages, which often cause compartment syndromes. Hemarthrosis is rare.
- Patients with acquired inhibitors are low responders.
- Transfusion therapy to achieve hemostasis is identical to the treatment of hemophiliacs with inhibitors.
- In contrast to hemophiliacs, most patients with spontaneous inhibitors respond to treatment to eradicate the inhibitor.
- Prednisone 1 mg/kg and oral cyclophosphamide 1 to 2 mg/kg daily have been used separately or in combination with high response rates.
- Intravenous immune globulin 1 g/kg daily for 2 days has also been shown to decrease inhibitor titers in some of these patients. Anecdotal successes after treatment with rituximab have been reported.

HEMOPHILIA B

Clinical Features

- Table 78–1 shows a clinical classification of hemophilia B based on factor IX levels.
- Bleeding episodes are clinically identical to those in hemophilia A.
- Factor IX inhibitors develop infrequently.

Laboratory Features

- In most cases, the aPTT is prolonged.
- Specific assay of factor IX levels is necessary for diagnosis.

Carrier Detection and Prenatal Diagnosis

- As with hemophilia A, molecular genetic techniques are available for carrier detection and prenatal diagnosis.

Differential Diagnosis

- Hemophilia B must be distinguished from hemophilia A, inherited or acquired deficiencies of other vitamin K–dependent coagulation factors, liver disease, or warfarin overdosage.

Treatment

- General treatment should be the same as for hemophilia A (see above).
- Replacement with factor IX concentrates (**Table 78–5**):
  — All currently available factor IX concentrates are treated to inactivate viruses.
  — Intermediate purity products (“prothrombin complex concentrates”) contain prothrombin; factors VII, IX, and X; and proteins C and S. They may also contain small amounts of activated factors VII, IX, and X, which predispose to thrombosis, especially if large doses are given or the patient has liver disease. Some concentrates contain traces of heparin.
  — Highly purified factor IX concentrates contain only traces of other prothrombin complex factors, and recombinant factor IX contains none. These are the currently preferred

preparations for clinical use.
  - — Intravascular recovery of factor IX from concentrates is about 50%, and is even less with the recombinant product.
  - — Initial dosage can be calculated assuming 1 unit of highly purified factor IX per kg body weight will increase the plasma level of factor IX by 1% or 0.01 U/mL. Thus, to replace factor IX to 100% requires 100 U/kg body weight as a bolus. The half-life of factor IX is 18 to 24 hours. Continued dosage should be one-half of the initial dosage given every 12 to 18 hours. Larger doses are required with recombinant factor IX.
  - — Factor IX may also be given by continuous infusion.
  - — Factor IX levels should be monitored during therapy and doses adjusted appropriately.
- Prophylactic therapy may also be given for hemophilia B. The recommended dose is 25 to 40 U/kg twice weekly.
- Gene therapy for hemophilia B is being actively investigated.

TABLE 78–5 CURRENTLY AVAILABLE FACTOR IX PRODUCTS*

	Origin	Viral Inactivation
Intermediate purity (prothrombin complex concentrates)		
Profilnine SD	Plasma	Solvent-detergent
Bebulin VH	Plasma	Vapor heating
High purity		
Mononine	Plasma	Ultra filtration; chemical
AlphaNine	Plasma	Solvent-detergent; virus filtered
Recombinant		
BeneFIX	CHO cells	Solvent-detergent Nanofiltration
Rixubis	CHO cells	Solvent-detergent Nanofiltration
Alprolix	HEK cells	Nanofiltration Chromatography

*Additional factor IX concentrates are available in Europe.
Source: *Williams Hematology*, 9th ed, Chap. 123, Table 123–7.

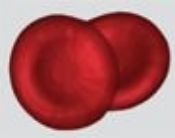
Course and Prognosis

- Patients with hemophilia B are vulnerable to the same complications of recurrent bleeding that occur with hemophilia A.
- HIV infection and chronic liver disease are common in patients treated before viral inactivation of factor IX concentrates was introduced.

Factor IX Inhibitors

- Factor IX inhibitors at levels less than 10 BU can sometimes be overcome with large doses of purified factor IX concentrates.
- If the factor IX inhibitor level is greater than 10 BU, inhibitor-bypassing products (recombinant factor VIIa or prothrombin complex concentrates) should be used.

- Attempts to induce immune tolerance by administering daily infusions of factor IX concentrates have led to significant adverse reactions, including anaphylaxis and the nephrotic syndrome. Factor VIIa concentrates should be used for treatment of any patient who has developed anaphylaxis.



For a more detailed discussion, see Miguel A. Escobar and Nigel S. Key: Hemophilia A and Hemophilia B, Chap. 123 in *Williams Hematology*, 9th ed.

CHAPTER 79
von Willebrand Disease

The condition known as von Willebrand disease (VWD) is a result of quantitative and qualitative abnormalities in von Willebrand factor (VWF), a plasma protein serving as a carrier for factor VIII and as an adhesive link between platelets and damaged blood vessel walls. **Table 79–1** presents the nomenclature used in discussing the functions of VWF.

TABLE 79–1 VON WILLEBRAND FACTOR AND FACTOR VIII TERMINOLOGY

Factor VIII
Antihemophilic factor, the protein that is reduced in plasma of patients with classic hemophilia A and von Willebrand disease and is measured in standard coagulation assays
Factor VIII activity (factor VIII:C)
The coagulant property of the factor VIII protein (this term is sometimes used interchangeably with factor VIII)
Factor VIII antigen (VIII:Ag)
The antigenic determinant(s) on factor VIII measured by immunoassays, which may employ polyclonal or monoclonal antibodies
von Willebrand factor (VWF)
The large multimeric glycoprotein that is necessary for normal platelet adhesion, a normal bleeding time, and stabilizing factor VIII
von Willebrand factor antigen (VWF:Ag)
The antigenic determinant(s) on VWF measured by immunoassays, which may employ polyclonal or monoclonal antibodies; *inaccurate designations of historical interest only* include factor VIII-related antigen (VIIIR:Ag), factor VIII antigen, AHF antigen, and AHF-like antigen
Ristocetin cofactor activity (or: von Willebrand factor activity; VWF:act)
The property of VWF that supports ristocetin-induced agglutination of washed or fixed normal platelets
von Willebrand factor collage-binding activity (VWF:CB)
The property of VWF that supports binding to collage, measured by enzyme-linked immunosorbent assay (ELISA)

Source: *Williams Hematology,* 9th ed, Chap. 126, Table 126–1.

ETIOLOGY AND PATHOGENESIS

- VWF is synthesized in endothelial cells and megakaryocytes.
- Post-translational modification of the molecule involves glycosylation, sulfation, and multimer formation through extensive disulfide bond formation.
- VWF is stored in platelets and in Weibel-Palade bodies in endothelial cells.
- Secretion of VWF from Weibel-Palade bodies is both constitutive and regulated. High-molecular-weight multimers with the greatest activity are released in response to agents such as thrombin in vitro or desmopressin (DDAVP) in vivo.

- A specific VWF-processing protease can reduce the size of high-molecular-weight multimers in plasma.
- VWF plays an important role in platelet aggregation at sites of vessel injury.
- VWF stabilizes factor VIII through formation of a noncovalent complex between the two proteins.
- A large number of mutations of the VWF gene have been discovered, and more than 20 distinct subtypes of VWD have been described. **Table 79–2** presents a simplified classification of VWD.
- Types 1 and 3 are deficiencies of normal VWF, either partial (type 1) or complete (type 3).
- Type 2 includes the qualitative abnormalities of VWF structure and/or function. The quantity of VWF (VWF antigen) in type 2 disease may be normal but is usually reduced.
- Platelet-type VWD is an inherited platelet abnormality due to a mutation in glycoprotein Ib (CD42b, c). It is discussed in Chap. 75.

TABLE 79–2 CLASSIFICATION OF VON WILLEBRAND DISEASE

Type	Molecular Characteristics	Inheritance	Frequency	Factor VIII Activity	VWF Antigen	Ristocetin Cofactor Activity	RIPA	Plasma VWF Multimer Structure
Type 1	Partial quantitative VWF deficiency	Autosomal dominant, incomplete penetrance	1–30:1000; most common VWD variant (> 70% of VWD)	Decreased	Decreased	Decreased	Decreased or normal	Normal distribution (mutant subunits permitted)
Type 3	Severe quantitative reduction or absence of VWF	Autosomal recessive (or codominant)	1–5:1,000,000	Markedly decreased	Very low or absent	Very low or absent	Absent	Usually absent
Type 2A	Qualitative VWF defect; loss of large VWF multimers, decreased VWF-dependent platelet adhesion	Usually autosomal dominant	~10%–15% of clinically significant VWD	Decreased to normal	Usually low	Markedly decreased	Decreased	Largest and intermediate multimers absent
Type 2B	Qualitative VWF defect; increased VWF–platelet interaction (GPIb)	Autosomal dominant	Uncommon variant (< 5% of clinical VWD)	Decreased to normal	Usually low	Decreased to normal	Increased to low concentrations of ristocetin	Largest multimers reduced/absent
Type 2M	Qualitative VWF defect; decreased VWF-platelet interaction, no loss of large VWF multimers	Usually autosomal dominant	Rare (case reports)	Variably decreased	Variably decreased	Decreased	Variably decreased	Normal and occasionally ultralarge forms
Type 2N	Qualitative VWF defect; decreased VWF-factor VIII binding capacity	Autosomal recessive	Uncommon; heterozygotes may be prevalent in some populations	Decreased	Normal	Normal	Normal	Normal
Platelet-type (pseudo-)	Platelet defect; decreased platelet-VWF interactions	Autosomal dominant	Rare	Decreased to normal	Decreased to normal	Decreased	Increased to low concentrations of ristocetin	Largest multimers absent

GPIb, glycoprotein Ib; RIPA, ristocetin-induced platelet aggregation; VWD, von Willebrand disease; VWF, von Willebrand factor.
Source: *Williams Hematology*, 9th ed, Chap. 126, Table 126–2.

CLINICAL FEATURES

Type 1

- Type 1 accounts for 70% of cases.
- It is usually transmitted as an autosomal dominant trait with variable expression and incomplete penetrance (heterozygous defect).
- Symptoms vary considerably in families. In two families, only 65% of individuals with both an affected parent and descendant had significant symptoms.
- Symptoms may vary in the same patient over time.

- The most common bleeding problems are epistaxis (60%), easy bruising and hematomas (40%), menorrhagia (35%), gingival bleeding (35%), and gastrointestinal bleeding (10%).
- In some families, there may be an association with hereditary hemorrhagic telangiectasia.
- Bleeding after trauma is common.
- Hemarthroses are rare except in association with trauma.
- In patients with mild to moderate disease, symptoms may ameliorate by the second or third decade of life.
- During pregnancy in patients with type 1 VWD, levels of factor VIII and ristocetin cofactor activities usually rise above 50%.

Type 2

- Types 2A and 2B are the most common qualitative VWF disorders. In type 2A, VWF function is impaired. In type 2B, the interaction between VWF and platelets is dysfunctional.
- Type 2 variants are usually transmitted as autosomal dominant traits. They account for 20% to 30% of cases.
- Thrombocytopenia occurs in type 2B but is usually not sufficiently severe to contribute to clinical bleeding.
- Infants with type 2B may have neonatal thrombocytopenia.
- Type 2N patients (with impaired factor VIII binding to VWF) usually have moderately decreased levels of factor VIII but may have low levels compatible with severe hemophilia A.

Type 3

- Inheritance may be autosomal recessive (homozygous or compound heterozygous defect).
- Major clinical bleeding, including hemarthroses and muscle hematomas, occurs as in severe hemophilia.

LABORATORY FEATURES

- In a patient suspected of having VWD, initial laboratory tests should include assay of VWF activity, VWF antigen, and factor VIII activity.
- Additional tests commonly performed are platelet function analysis tests by automated machines, ristocetin-induced platelet agglutination, and VWF multimer analysis (**Figure 79–1**).
- Factor VIII activity, VWF antigen, and ristocetin cofactor activity may all be increased to normal by many minor illnesses.
- VWF levels may vary with blood group. Carriers of blood group O typically have lower VWF levels.
- Wide variation is found in the results of repeated assays for VWF or ristocetin cofactor activity in the same subjects.
- Repeated studies are usually necessary, and the diagnosis or exclusion of VWD usually requires more than one set of laboratory data.

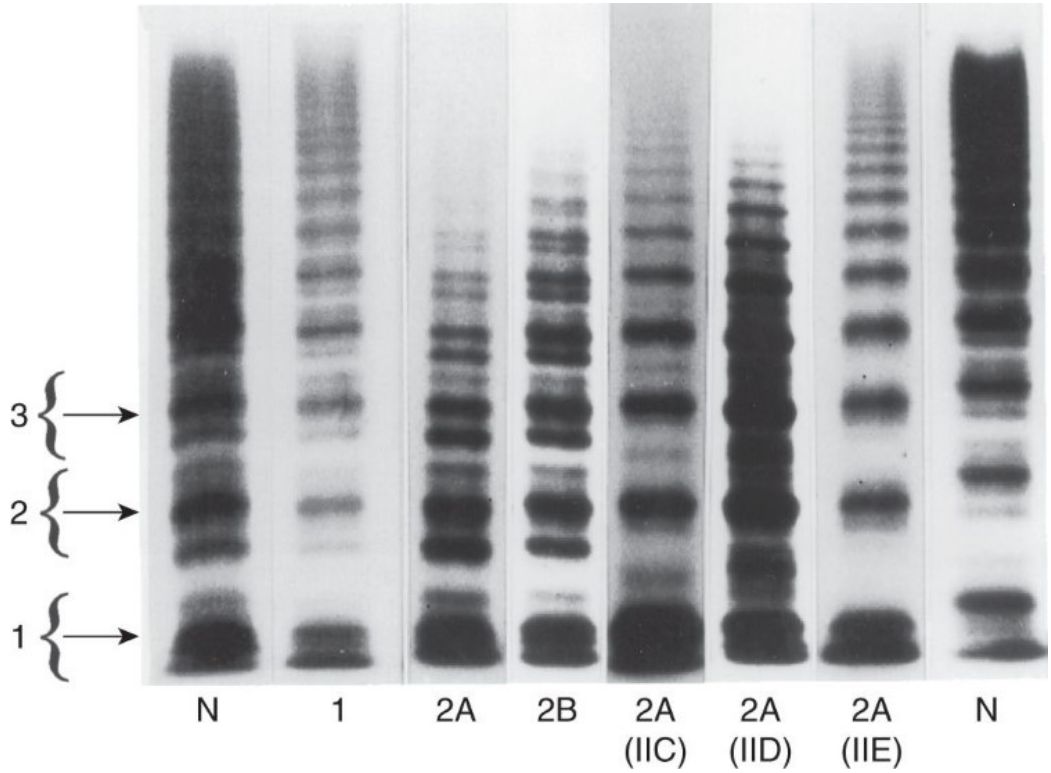


FIGURE 79–1 Agarose gel electrophoresis of plasma VWF. VWF multimers from plasma of patients with various subtypes of VWD are shown. The brackets to the left encompass three individual multimer subunits, including the main band and its associate satellite bands. N indicates normal control lanes. Lanes 5 through 7 are rare variants of type 2A VWD. The former designations for these variants are indicated in parentheses below the lanes (IIC through IIE). (Reproduced with permission from Zimmerman TS, Dent JA, Ruggeri ZM, Nannini LH: Subunit composition of plasma von Willebrand factor. Cleavage is present in normal individuals, increased in IIA and IIB von Willebrand disease, but minimal in variants with aberrant structure of individual oligomers (types IIC, IID, and IIE). *J Clin Invest* Mar;77(3):947–51, 1986.)

DIFFERENTIAL DIAGNOSIS

Prenatal Diagnosis

- In most instances, the clinical phenotype of VWD is mild and prenatal diagnosis is rarely sought.
- Prenatal diagnosis has been successful using DNA techniques in families with type 3 VWD.

Platelet-Type (Pseudo-) VWD

- This is a platelet defect discussed in Chap. 75. It can be differentiated from VWD by special laboratory tests.

Acquired VWD

- Acquired VWD usually appears later in life in a patient with no personal or family history of abnormal bleeding.
- Another disease is usually present, such as essential thrombocythemia, hypothyroidism, a benign or malignant B-cell disorder, a solid tumor, or a cardiac or vascular defect.
- Several drugs, including ciprofloxacin and valproic acid, have been associated with acquired VWD.
- The patients have decreased levels of factor VIII, VWF antigen, and ristocetin cofactor

activity. Large multimers of VWF are relatively depleted from the plasma. The bleeding time is usually prolonged.

- Autoantibodies to VWF appear to be responsible for the disease in most instances, usually by causing rapid clearance of VWF from the circulation but sometimes by interfering with VWF function.
- Reduced levels of VWF may also be caused by decreased synthesis (eg, hypothyroidism), increased destruction (eg, heart disease, some drugs), or selective adsorption to tumor cells.
- Laboratory confirmation of acquired VWD can be very difficult, and the diagnosis may depend on the late onset, absence of personal or family bleeding history, and identification of the underlying disease.
- Management is usually directed to the underlying disorder. Refractory patients have been treated with glucocorticoids, plasma exchange, or intravenous immunoglobulin (IVIG).
- Bleeding in patients with acquired VWD can be managed by (high dose) VWF concentrate, DDAVP, or by recombinant factor VIIa.

TREATMENT, COURSE, PROGNOSIS

- The goals of therapy are to correct the VWF deficiency and shorten or correct the bleeding time.

Desmopressin

- Patients with type 1 VWD release unusually high-molecular-weight multimers of VWF into the circulation for 1 to 3 hours after infusion of desmopressin (DDAVP).
- Therapy with DDAVP increases the baseline levels of factor VIII activity, VWF antigen, and activity two- to five-fold in patients with type 1 VWD, and in many instances also corrects the abnormal bleeding time.
- Approximately 80% of type 1 patients have excellent responses to DDAVP. Many type 2 patients and nearly all type 3 do not respond adequately.
- DDAVP is regularly used in patients with type 1 VWD to treat mild to moderate bleeding, or as prophylaxis prior to surgery.
- Patients being considered for DDAVP therapy should, if possible, have factor VIII and ristocetin cofactor levels determined 1 to 2 hours following a preliminary dose.
- For patients undergoing surgery, DDAVP can be given 1 hour prior to the operation and repeated every 12 hours. The usual dose is 0.3 µg/kg in 100 mL saline over 30 to 45 minutes intravenously.
- Alternatively, nasal spray may be used (fixed dose of 300 mcg for adults and 150 mcg for children). The effect on VWF is somewhat more variable.
- Mild cutaneous vasodilatation is common, leading to facial flushing, tingling, warmth, and headaches.
- Fluid restriction may be necessary because of the potential for dilutional hyponatremia, in particular in children and perioperative patients.
- There have been isolated reports of arterial thrombosis (including myocardial infarction and unstable angina) with DDAVP therapy.

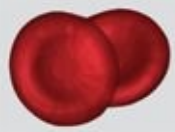
- Response to DDAVP may be reduced in patients receiving doses more frequently than every 24 to 48 hours (tachyphylaxis).
- The response of factor VIII level and ristocetin cofactor activity should be measured regularly in patients receiving frequent doses of DDAVP.
- VWF-containing concentrates and/or cryoprecipitate should be available for use in the event that DDAVP becomes ineffective.
- DDAVP has been successfully used to treat type 2B patients, but there is concern that the release of high-molecular-weight multimers could cause platelet aggregation and worsening thrombocytopenia in some patients.

VWF Replacement

- Patients unresponsive to DDAVP may be treated with virus-inactivated, VWF-containing factor VIII concentrates, such as Humate P.
- Replacement therapy is largely empiric, with the initial goal normalization of factor VIII levels and shortening or normalization of the bleeding time.
- If clinical bleeding continues, additional replacement should be given and the patient evaluated for other causes of bleeding that may require additional intervention.
- Patients should be treated for 7 to 10 days after major surgical procedures and 3 to 5 days after minor.
- Postpartum bleeding may occur for more than a month after delivery and may require prolonged treatment in some severe cases.
- Patients with type 3 VWD may develop an autoantibody against VWF, requiring treatment similar to that of factor VIII inhibitors in hemophilia A.

Other Treatments

- Estrogens or oral contraceptives have been used empirically for menorrhagia.
- Fibrinolytic inhibitors, such as ε-aminocaproic acid and tranexamic acid, may be useful adjuncts to prophylactic therapy for dental procedures, and have also been used empirically in menorrhagia or recurrent epistaxis.



For a more detailed discussion, see Jill Johnsen and David Ginsburg: von Willebrand Disease, Chap. 126 in *Williams Hematology*, 9th ed.

CHAPTER 80

Hereditary Disorders of Fibrinogen

AFIBRINOGENEMIA AND HYPOFIBRINOGENEMIA

- Quantitative disorders of fibrinogen may be afibrinogenemia or hypofibrinogenemia, depending on the severity.
- Normal fibrinogen levels range from 150 to 350 mg/dL. In afibrinogenemia, the fibrinogen concentration is less than 20 mg/dL. In hypofibrinogenemia, the level is less than normal.
- Approximately 100 distinct mutations have been identified in patients with afibrinogenemia (in homozygosity or in compound heterozygosity) or in hypofibrinogenemia. Causative mutations can be divided into two main classes: null mutations with no protein production at all and mutations producing abnormal protein chains that are retained inside the cell.

Clinical Features

- Congenital afibrinogenemia is a rare disorder of hepatic biosynthesis of fibrinogen, inherited as an autosomal recessive trait, with low levels of fibrinogen typically found in both parents.
- Bleeding varies from minimal to severe. Umbilical cord bleeding may occur at birth. Later, bleeding may be from mucosal surfaces, into muscles, or into joints.
- Spontaneous abortions are frequent.
- Death is most often a result of intracranial hemorrhage.
- Hereditary hypofibrinogenemia appears to be caused by abnormal intracellular hepatic storage of fibrinogen.

Laboratory Features

- All laboratory tests depending on formation of a clot are abnormal in afibrinogenemia or hypofibrinogenemia but can be corrected by mixing with normal plasma or fibrinogen solutions.
- The diagnosis is established by demonstrating a reduced fibrinogen concentration by immunologic testing.
- The bleeding time is prolonged and platelet aggregation is abnormal; both can be corrected by infusion of plasma or fibrinogen.

Treatment, Course, and Prognosis

- Replacement therapy with cryoprecipitate (if available) or fibrinogen concentrate may be required.
- Cryoprecipitate typically contains 300 mg of fibrinogen per unit. Approximately 50% to 70% percent of the administered fibrinogen circulates post-transfusion, and the biologic half-life of

fibrinogen is 3 to 5 days. The recommended initial dose is 1 unit of cryoprecipitate (300 mg of fibrinogen) per 5 kg of body weight to reach hemostatic levels of fibrinogen.

- Fibrinogen concentrate should be given to increase the plasma concentrations by at least 150 mg/dL. One gram of fibrinogen concentrate raises the plasma fibrinogen level by 20 mg/dL.
- Patients should receive one-third of the initial loading dose daily as long as is necessary to sustain the fibrinogen level.
- Cryoprecipitate or fibrinogen concentrate may be given during pregnancy to prevent spontaneous abortion or premature birth.
- Thrombosis can occur after administration of fibrinogen, and antifibrinogen antibodies may develop.

DYSFIBRINOGENEMIA

- Inherited dysfibrinogenemia is the production of structurally abnormal fibrinogen molecules with altered functional properties. At least 300 families with this fibrinogenemia have been described thus far.
- Hypodysfibrinogenemia refers to patients with low levels of circulating abnormal fibrinogen.

Etiology and Pathogenesis

- Dysfibrinogenemia is inherited as an autosomal dominant trait. Most patients are heterozygous but some are homozygous.
- Fibrinogen abnormalities usually affect one or more phases of fibrin formation:
  — Impaired fibrinopeptide release
  — Defective fibrin polymerization
  — Defective cross-linking by factor XIIIa
- Biochemical abnormalities do not correlate with clinical expression. For example, the same amino acid substitution can lead to either a familial bleeding tendency or to a familial thrombophilia.
- Hereditary renal amyloidosis is an autosomal dominant trait in which there is progressive extracellular deposition of “amyloid” protein in the kidneys, due in some instances, to deposition of fragments of a structurally abnormal fibrinogen.
- More than 100 distinct mutations have been identified in patients with dysfibrinogenemia and hypodysfibrinogenemia. The described mutants are very often named after the city of origin of the family or the city of the laboratory characterizing the mutation.

Clinical Features

- Most patients are asymptomatic (identified as a result of routine coagulation screening). About 25% have abnormal bleeding, and 20% have thrombophilia. Some patients have both thrombophilia and bleeding.
- Bleeding is usually not severe (eg, epistaxis, menorrhagia, mild to moderate postoperative hemorrhage).
- Spontaneous abortion may occur, and excessive bleeding or thromboembolism may be seen postpartum.

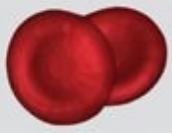
- Defective wound healing occurs with several variants.
- Thrombosis is usually venous but may be arterial.
- Renal amyloidosis occurs in some families.

Laboratory Features

- Coagulation tests requiring the formation of a fibrin clot are usually prolonged (eg, prothrombin time, activated partial thromboplastin time, thrombin time).
- Some variants may be detected only by abnormalities of the thrombin and/or reptilase times. It is essential to compare fibrinogen concentrations determined by different methods: functional, immunologic, and chemical. The diagnosis is based on an abnormally low functional fibrinogen level, with a normal level by immunologic or chemical methods. In hypodysfibrinogenemia, reduced levels are found by all three methods. Here the diagnosis must be made from abnormal thrombin and reptilase times.
- Impaired platelet aggregation and clot retraction have been reported in some families. In one family, enhanced platelet aggregation has been described.

Treatment

- Patients with bleeding or undergoing surgery may require replacement therapy with cryoprecipitate or fibrinogen concentrate as outlined for afibrinogenemia.
- Thromboembolism is treated with anticoagulants following standard protocols.
- Administration of cryoprecipitate or fibrinogen concentrate prior to surgery may be beneficial both to increase the level of normal fibrinogen and to dilute the prothrombotic fibrinogen. In patients with life-threatening thromboembolic disease undergoing surgery, plasma exchange has been effective.



For a more detailed discussion, see Marguerite Neerman-Arbez and Phillipe de Moerloose: Hereditary Fibrinogen Abnormalities, Chap. 125 in *Williams Hematology*, 9th ed.

CHAPTER 81

Inherited Deficiencies of Coagulation Factors II, V, V + VIII, VII, X, XI, and XIII

- Inherited deficiencies of coagulation factors other than factor VIII (hemophilia A) and factor IX (hemophilia B) are rare bleeding disorders that occur in most populations.
- Patients are usually homozygotes or compound heterozygotes.
- Factor XI and factor VII deficiency occur relatively frequently, and other deficiencies are relatively rare (Table 81–1).
- The severity of the bleeding disorder usually relates to the severity of the factor deficiency.
- All may be caused by decreased synthesis of a specific coagulation factor, by synthesis of a dysfunctional form of the coagulation factor, or both.
- Inherited deficiency of a coagulation factor does not protect patients from thrombosis.

TABLE 81–1 RELATIVE PREVALENCE OF RARE BLEEDING DISORDERS*

	WFH Survey (2002)†		Six National Registries (2007)†		UK Data (Oct. 2008)‡		Survey of 64 Centers (Aug. 2008)†	
Deficiency	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Factor XI	2446	35.3	1947	39.4	1762	59.5	770	23.5
Factor VII	1689	24.4	1050	21.3	580	19.6	927	28.3
Afibrinogenemia	644	9.3	496	10.0	203	6.9	241	7.4
Factor X	597	8.6	446	9.0	190	6.4	339	10.4
Factor V	769	11.1	415	8.4	129	4.4	233	7.1
Factor XIII	434	6.3	282	5.7	60	2.0	211	6.5
Factor V/Factor VIII	188	2.7	203	4.1	25	0.8	495	15.1
Factor II	167	2.4	101	2.0	13	0.4	55	1.7
Total	6934	100	4940	100	2962	100	3271	100

*Patients with partial deficiency were included.

†Data courtesy of Professor Flora Peyvandi, Milan, Italy.

‡Data courtesy of Professor Paula Bolton-Maggs, Manchester, UK.

Source: *Williams Hematology,* 8th ed, Chap. 125, Table 125–1.

PROTHROMBIN (FACTOR II) DEFICIENCY

Pathogenesis

- Hypoprothrombinemia or dysprothrombinemia may be involved.

- Both are inherited as autosomal recessive disorders.
- Both interfere with hemostasis by impairing thrombin generation.

Clinical Features

- The disorders are characterized by mucocutaneous and soft-tissue bleeding, usually in proportion to the severity of the functional prothrombin deficiency.
- Bleeding may be spontaneous if prothrombin levels are less than 1%. Hemarthroses may occur.
- Individuals with higher prothrombin levels have a variable bleeding tendency, and some may be asymptomatic.

Laboratory Features

- The activated partial thromboplastin time (aPTT) and prothrombin time (PT) are prolonged. The thrombin time (TT) is normal.
- Diagnosis is established by demonstrating reduced levels of functional prothrombin.
- Both functional and antigen assays are required to identify dysprothrombinemia. Immunoelectrophoretic studies may demonstrate some forms of dysprothrombinemia.

Differential Diagnosis

- Differential diagnosis includes inherited factor V or factor X deficiency, acquired deficiency of the vitamin K–dependent factors, or lupus anticoagulant.

Treatment

- Prothrombin deficiency may be corrected with intravenous prothrombin complex concentrates, but with risk of transmission of viruses not inactivated by solvent detergent treatment and/or nanofiltration and induction of intravascular coagulation.
- Fresh-frozen plasma is also effective but carries a risk of transmitting infectious agents. Solvent detergent treatment of pooled plasma reduces this risk, but viruses that are not inactivated in the pooled plasma source may still be transmitted (eg, parvovirus, hepatitis A virus).
- Bruises and mild superficial bleeding do not require treatment.
- The biologic half-life of prothrombin is 3 days, and a single treatment for a bleeding episode may suffice.
- Prothrombin levels of 10% to 25% are usually sufficient for hemostasis.

FACTOR V DEFICIENCY

Pathogenesis

- Inherited factor V deficiency is transmitted as an autosomal recessive disorder.
- Homozygotes have a moderate bleeding tendency that is usually due to a true deficiency, but the disorder may also be caused by dysfunctional factor V.
- Heterozygotes are usually asymptomatic.

Clinical Features

- Patients with 1% to 10% factor V activity have lifelong bleeding, usually expressed as ecchymoses, epistaxis, gingival bleeding, excessive bleeding from minor lacerations, and menorrhagia.
- Hemarthroses or intracranial hemorrhage has been reported.
- Severe bleeding may occur after trauma, dental extraction, or surgery.

Laboratory Features

- Factor V deficiency is characterized by prolongation of both the aPTT and the PT.
- Diagnosis requires specific demonstration of a factor V deficiency.

Differential Diagnosis

- The clinical and laboratory features of hereditary combined factor V and factor VIII deficiency are the same as those of factor V deficiency. Specific assay for factor VIII deficiency is needed to differentiate these diseases.
- The clinical features of severe liver disease or disseminated intravascular coagulation (DIC) are usually sufficient to permit diagnosis of this cause of acquired factor V deficiency.
- Acquired inhibitors of factor V may appear rarely after surgery or during therapy with antibiotics or other drugs, and they can cause severe bleeding. These inhibitors often disappear spontaneously.

Treatment

- Severe or continuing mild bleeding is treated with replacement therapy using fresh-frozen plasma. A factor V level of 25% is usually sufficient for hemostasis. The plasma factor V half-life is 12 to 14 hours.
- Infusion of a loading dose of 20 mL/kg of fresh-frozen plasma followed by 5 to 10 mL/kg every 12 hours for 7 to 10 days is usually adequate to ensure hemostasis.
- Minor lacerations may be treated with local measures.
- Antifibrinolytic therapy may be effective in epistaxis or gingival bleeding.

COMBINED DEFICIENCY OF FACTORS V AND VIII

- A rare, autosomal recessive trait with reduced levels of both factor V and factor VIII expressed as a moderately severe lifelong bleeding disorder.
- The molecular basis of this condition relies on null mutations in the endoplasmic reticulum–Golgi intermediate compartment *(ERGIC)-53* gene, now called the *LMAN1* gene.
- Diagnosis requires specific assays of both factor V and factor VIII.
- Minor bleeding may respond to antifibrinolytic therapy.
- For severe bleeding or prophylaxis before surgery or dental extraction, replacement of both factor V, using fresh-frozen plasma, and factor VIII, using a factor VIII concentrate, is required.

FACTOR VII DEFICIENCY

Pathogenesis

- Factor VII deficiency is inherited as an autosomal recessive trait.
- The disorder is symptomatic only in homozygotes or compound heterozygotes.
- The disease may be caused by decreased production of factor VII, production of a factor VII with decreased functional activity, or both. Levels of factor VII antigen may be normal, reduced, or zero.
- Three polymorphisms of the factor VII gene that lead to reduced levels of factor VII but do not lead to abnormal bleeding have been described. These reduced levels of factor VII may be of benefit by lowering the risk of myocardial infarction.

Clinical Features

- Patients with factor VII levels below 1% may have a severe bleeding disorder indistinguishable from severe hemophilia A or B.
- Most patients with levels of factor VII of 5% or more have disease characterized by easy bruising, gingival bleeding, epistaxis, and menorrhagia.
- Dental extractions, tonsillectomy, and genitourinary tract surgery may induce excessive bleeding if no preoperative replacement therapy is given, but operations such as laparotomy and herniorrhaphy may not lead to excessive bleeding.
- Postpartum hemorrhage is unusual in women with factor VII deficiency.

Laboratory Features

- The diagnosis is suggested by a prolonged PT with a normal aPTT.
- Diagnosis requires demonstration of isolated factor VII deficiency by specific assay.
- Factor VII antigen can be detected by radioimmunoassay.
- The mutant gene can be detected by molecular biology techniques.

Differential Diagnosis

- Acquired factor VII deficiency occurs in patients with liver disease, vitamin K deficiency, and those receiving vitamin K antagonists.
- Rarely, patients may have an inherited deficiency of factor VII and X, factor VII and IX, or of all vitamin K–dependent factors.

Treatment

- Skin lacerations require only local hemostasis. Antifibrinolytic therapy is usually effective in patients with menorrhagia, epistaxis, and/or gingival bleeding.
- Replacement therapy is necessary in patients with severe bleeding, such as hemarthroses or intracerebral hemorrhage, and may be required with surgery, depending on the severity of the deficiency, bleeding history, and the operative site.
- Replacement may be achieved with plasma, prothrombin complex concentrates, specific factor VII concentrates, or recombinant human factor VIIa.
- The possibilities for transmission of viral infection and induction of thrombosis must be considered when selecting a therapeutic agent.
- The half-life of factor VII is approximately 5 hours. Hemostasis is achieved with levels

between 10% and 25%.
- If plasma is used for major surgery, the recommended initial dose is 15 mL/kg, followed by 4 mL/kg every 6 hours for 7 to 10 days.
- Replacement therapy with plasma may lead to fluid overload requiring diuretic therapy or plasmapheresis.

FACTOR X DEFICIENCY

Pathogenesis

- Factory X deficiency is inherited as an autosomal recessive trait.
- Heterozygotes have factor X levels about 50% of normal and are usually asymptomatic.
- The disease may be caused by decreased production of factor X, production of factor X with decreased functional activity, or both.

Clinical Features

- Patients with factor X levels of less than 1% have severe bleeding, primarily in the joints, soft tissues, and from mucous membranes. Menorrhagia may be a major problem.
- In patients with mild to moderate factor X deficiency, bleeding usually occurs after trauma or surgery.

Laboratory Features

- The PT and aPTT are both prolonged, as is the Russell viper venom time. The TT is normal.
- Diagnosis requires demonstration of isolated factor X deficiency by specific assay.
- Factor X antigen can be detected by immunologic techniques.

Differential Diagnosis

- Laboratory testing will differentiate inherited factor X deficiency from deficiency of prothrombin, factor V, factor VII, multiple factor deficiencies, vitamin K deficiency, liver disease, or the lupus anticoagulants.
- Acquired factor X deficiency may occur in patients with primary amyloidosis due to selective binding of factor X to amyloid fibrils or to the presence of an abnormal form of factor X.
- Acquired isolated factor X deficiency has been reported to be associated with a number of other disorders. Acquired inhibitors of factor X also occur.

Treatment

- Factor X deficiency may be treated with prothrombin complex concentrates that contain factor X. Because of the (theoretical) risk of thrombosis with these concentrates, it is recommended that divided doses be used if more than 2000 units are required.
- For soft tissue, mucosal, or joint hemorrhages, replacement of factor X to 30% of normal is recommended. More serious bleeding requires replacement to 50% to 100%.
- The biologic half-life of factor X is 24 to 40 hours. Continuing therapy should be given every 24 hours.

- Fresh-frozen plasma may also be used to replace factor X deficiency but carries the risks of viral infection and fluid overload.

FACTOR XI DEFICIENCY

Pathogenesis

- Factor XI deficiency is an autosomal recessive disorder caused by deficient production of factor XI in almost all instances.
- Homozygotes or compound heterozygotes have factor XI levels of less than 15% of normal.
- Factor XI is essential for the activation by thrombin of thrombin-activatable fibrinolysis inhibitor (TAFI) or carboxypeptidase B, an enzyme that inhibits fibrinolysis. This may result in increased fibrinolytic activity, with consequent increase in bleeding.

Clinical Features

- Most patients with factor XI deficiency are Jewish.
- Bleeding is usually related to trauma or surgery.
- Excessive bleeding may begin at the time of injury or be delayed for several hours.
- There appears to be a greater bleeding tendency in genotypes with lower levels of factor XI, and with surgery or injury at sites of high fibrinolytic activity, such as the urinary tract, tonsils, nose, or tooth sockets.
- Some patients who are heterozygous for factor XI deficiency may have excessive bleeding.
- Inhibitors of factor XI may develop in deficient patients who have received replacement therapy, but these do not appear to increase the risk of bleeding in most such patients.

Laboratory Features

- The aPTT is prolonged; the PT is normal.
- Diagnosis requires specific demonstration of a factor XI deficiency.
- The patient’s genotype can be determined by molecular biology techniques.

Treatment

- Patients with severe factor XI deficiency may be given replacement therapy with fresh-frozen plasma, recognizing the attendant risk of transmission of infectious agents or allergic reactions. Alternatively, in some countries (plasma-derived) purified and virus-inactivated factor XI concentrates are available.
- The mean half-life of factor XI is about 48 hours.
- Trough levels of factor XI of 45% maintained for 10 to 14 days provide adequate hemostasis after major surgery or surgery at sites with high fibrinolytic activity.
- Surgery in areas of lower fibrinolytic activity requires factor XI trough levels of 30% maintained for 5 to 7 days.
- Antifibrinolytic therapy may be effective in achieving hemostasis after dental extraction, and is a similarly useful adjunct for treating patients after operation on sites with high local fibrinolytic activity.
- Heterozygous patients with a negative bleeding history, no associated hemostatic abnormality,

and a factor XI level above 45% probably do not need treatment when undergoing surgery.
- Such individuals with a positive bleeding history and requiring surgery should have appropriate treatment of any associated disorder and replacement of factor XI to trough levels of 45% for 5 days.

FACTOR XIII DEFICIENCY

Pathogenesis

- Factor XIII deficiency is a lifelong bleeding disorder transmitted as an autosomal recessive trait.
- Factor XIII deficiency leads to clots that are less stable mechanically and more susceptible to fibrinolysis, resulting in the bleeding disorder.

Clinical Features

- Ecchymoses, hematomas, and prolonged post-traumatic bleeding are common.
- Bleeding from the umbilical cord of newborns occurs frequently.
- Intracranial hemorrhage occurs more often with factor XIII deficiency than with the other coagulation factor deficiencies when matched for the level of coagulation factor.
- Habitual abortion and poor wound healing also occur.

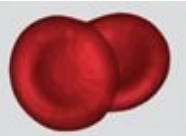
Laboratory Features

- Screening tests for coagulation abnormalities are all usually normal in factor XIII deficiency, although in some cases, the thrombin time may be minimally prolonged. The diagnosis is established by demonstrating increased clot solubility in 5-M urea or by chemical assays for factor XIIIa activity.
- Deficiency of α_2-antiplasmin gives a similar pattern as factor XIII deficiency but can be diagnosed by specific assay.
- Acquired factor XIII deficiency may occur in DIC, primary fibrinolysis, or if an inhibitor develops to factor XIII. Factor XIII levels may also be decreased after major surgery, during chronic inflammatory conditions (eg, inflammatory bowel disease), and major trauma.

Treatment

- Replacement therapy may be achieved with plasma or cryoprecipitate, with attendant risks of transmission of infectious agents, or with virus-inactivated concentrates of factor XIII from plasma, if available.
- Factor XIII levels of less than 5% will achieve hemostasis.
- The half-life of factor XIII is 19 days.
- Prophylactic therapy using plasma infusions every 4 weeks can achieve normal hemostasis and prevent habitual abortions.

For a more detailed discussion, see Flora Peyvandi and Marzia Menegatti: Inherited Deficiencies of Coagulation Factors II, V, V + VIII, VII, X, XI, and XIII, Chap. 124 in *Williams Hematology*, 9th ed.



CHAPTER 82

Antibody-Mediated Coagulation Factor Deficiencies

- Clinically significant autoantibodies to coagulation factors are uncommon but can produce life-threatening bleeding and death.
- The most commonly targeted coagulation factor by an autoantibody is factor VIII (acquired hemophilia A) but also any other coagulation factor may be inhibited by an autoantibody.

ACQUIRED HEMOPHILIA A

- Acquired hemophilia A can either be idiopathic or associated with other autoimmune disorders, malignancy, the postpartum period, and the use of drugs (such as penicillin and sulfonamides).
- The incidence of autoantibodies to factor VIII is 0.2 to 1 per 1 million persons per year.
- Acquired hemophilia A patients usually present with spontaneous bleeding, which often is severe and life- or limb-threatening. These patients are more likely to have a more severe bleeding diathesis than patients with hemophilia A and an inhibitor.
- Common bleeding sites are soft tissues, skin, and mucous membranes. In contrast to patients with congenital hemophilia A, hemarthroses, intramuscular, and central nervous system bleeding are rare.
- Patients with acquired hemophilia A have a prolonged activated partial thromboplastin time (aPTT) and a normal prothrombin time (PT). The presence of a prolonged aPTT in a 1:1 mixture between patient and normal plasma establishes the diagnosis of a circulating anticoagulant. Specific assays for factor VIII activity and/or antigen will confirm the diagnosis.
- Once the identity of an inhibitor has been established, its titer is determined using the Bethesda assay. The inhibitor titer is defined as the dilution of patient plasma that produces 50% inhibition of the factor VIII activity and is expressed as Bethesda units per mL (BU/mL). Inhibitors are classified as low titer or high titer when the titers are less than 5 BU/mL or greater than 5 BU/mL, respectively.
- Acquired factor VIII inhibitors sometimes resolve spontaneously. However, it is not possible to predict in which subset of patients this will occur, and so treatment will be required when bleeding complications ensue.
- Patients with a factor VIII inhibitor titer of less than 5 BU/mL often are treated successfully with sufficient doses of recombinant or plasma-derived factor VIII concentrates to neutralize the inhibitor. Patients with titers between 5 and 10 BU/mL also may respond to factor VIII concentrates, whereas those with titers greater than 10 BU/mL generally do not respond.
- Factor VIII bypassing agents, which drive the coagulation mechanism through the extrinsic pathway, are the mainstays of management of patients with a high titer of an inhibitor. Two

agents, recombinant activated factor VII and plasma-derived factor eight-inhibitor bypassing agent (FEIBA; also called activated prothrombin complex concentrate) are approved by the US Food and Drug Administration for treatment of acquired hemophilia A.

- The recommended dose range of rFVIIa for the treatment of patients with hemorrhage due to acquired hemophilia is 70 to 90 µg/kg repeated every 2 to 3 hours until hemostasis is achieved. The minimum effective dose in acquired hemophilia has not been determined.
- Recommended doses of FEIBA depend on the type of bleeding.
  - — In joint hemorrhage, 50 U/kg is recommended at 12-hour intervals, which may be increased to doses of 100 U/kg. Treatment should be continued until clear signs of clinical improvement appear, such as relief of pain, reduction of swelling, or mobilization of the joint.
  - — For mucous membrane bleeding, 50 U/kg is recommended at 6-hour intervals under careful monitoring. If hemorrhage does not stop, the dose may be increased to 100 U/kg at 6-hour intervals.
  - — For severe soft-tissue bleeding, such as retroperitoneal bleeding, 100 U/kg at 12-hour intervals is recommended.
  - — Central nervous system bleeding has been effectively treated with doses of 100 U/kg at 6- to 12-hour intervals. One should not exceed a daily dose of FEIBA of 200 U/kg.
- The response to bypassing agents is variable and does not correlate with the inhibitor titer. A major concern with the use of rFVIIa and activated coagulation factor concentrates is that there is no laboratory method available for predicting response to therapy or monitoring patients on therapy.
- The major serious adverse event associated with bypassing agents is thrombosis. However, this risk is considered low when used for approved indications at the recommended doses.
- A commercial plasma-derived porcine factor VIII concentrate was useful in the treatment of factor VIII inhibitor patients for approximately 20 years but was discontinued in 2004 because of viral contamination of the product. Porcine factor VIII has the advantage of potentially being guided by laboratory monitoring of recovery of factor VIII activity in plasma. However, the development of antiporcine factor VIII antibodies often precluded its long-term use.
- Although acquired inhibitors may remit spontaneously, initiation of immunosuppressive therapy at the time of diagnosis to eradicate the inhibitor is recommended because of the serious course of this condition. A variety of immunosuppressive agents have been used, including cyclophosphamide, azathioprine, cyclosporine A, intravenous immunoglobulin, and rituximab. Plasmapheresis and immunoadsorption of the inhibitory antibody have been used. Finally, immune tolerance induction using human factor VIII has been used successfully.

ANTI–FACTOR V AND ANTITHROMBIN ANTIBODIES

- Antibodies inhibiting thrombin and factor V frequently coexist in immune responses to commercial products that contain thrombin (eg, adhesive tissue glue). Thrombin products have been used widely in surgical and endoscopic procedures.
- Thrombin is used either alone or as a component of fibrin sealants, which consist of fibrinogen and thrombin preparations that are mixed together at the wound site to form a topical fibrin clot. Both types of products are heavily contaminated with other plasma proteins, including

factor V and prothrombin. Almost all patients exposed to bovine proteins develop a detectable immune response. In half of these patients, antibovine antibodies cross-react with human thrombin, factor V, or prothrombin.

- Usually, these antibodies cause no clinical problems. However, mild to life-threatening hemorrhage can occur, especially if the titer of antihuman factor V antibodies is high. The risk of bleeding is higher in patients who receive bovine thrombin products more than once because of the development of a secondary immune response.
- β-lactam antibiotics also have been associated with anti–factor V autoantibodies and may partly explain the increased incidence with surgery. Anti–factor V autoantibodies have been identified rarely in patients with autoimmune diseases, solid tumors, and monoclonal gammopathies. In approximately 20% of cases of factor V autoantibody formation, no underlying disease was identified.
- Patients with inhibitory antibodies to factor V have prolonged PT and aPTT, low factor V levels and a normal thrombin time. The diagnosis of a factor V inhibitor is based on the specific loss of factor V coagulant activity when patient and normal plasma are mixed in a coagulation assay.
- In case of bleeding, patients may be treated with (high dose) fresh frozen plasma or with a bypassing agent, such as recombinant factor VIIa.

ANTIPROTHROMBIN ANTIBODIES

- Antiprothrombin antibodies are most commonly associated with the antiphospholipid syndrome (see Chap. 84). The antiphospholipid syndrome is caused by lupus anticoagulants, which are defined as antibodies that produce phospholipid-dependent prolongation of in vitro coagulation assays.
- However, most patients with lupus anticoagulants have demonstrable antiprothrombin antibodies or a hypoprothrombinemia but no bleeding diathesis.

ACQUIRED ANTIBODIES TO OTHER COAGULATION FACTORS

- Clinically significant antibodies to coagulation factors other than factor VIII, factor V, and prothrombin that produce acquired bleeding disorders are rare. In contrast to acquired hemophilia A, acquired hemophilia B is extremely rare.
- An acquired inhibitor to protein C associated with a fatal thrombotic disorder has been reported, but evidently is rare.
- In contrast, there is a relatively high prevalence of pathogenic anti–protein S antibodies. Inhibitory antibodies to protein S were detected in 5 of 15 patients with acquired protein S deficiency. Anti–protein S antibodies appear to be a risk factor for venous thrombosis and can be manifested in vitro as activated protein C resistance.

For a more detailed discussion, see Sean R. Stowell, Pete Lollar, and Shannon L. Meeks: Antibody-Mediated Coagulation Factor Deficiencies, Chap. 127 in *Williams Hematology*, 9th ed.

CHAPTER 83

Hemostatic Dysfunction Related to Liver Diseases

PATHOGENESIS

- Loss of hepatic parenchymal cells leads to decreased plasma levels of all plasma coagulation factors except factor VIII and von Willebrand factor.
- Thrombocytopenia occurs frequently and is usually a result of splenic sequestration (see Chap. 73) but may also be caused by an autoimmune mechanism, disseminated intravascular coagulation (DIC), folic acid deficiency, and decreased platelet production. Thrombocytopenia due to thrombopoietin (TPO) deficiency and platelet dysfunction contribute to the hemostatic abnormalities.
- Enhanced fibrinolysis is common and appears to be caused by complex pathogenetic mechanisms, including release and impaired clearance of plasminogen activators.
- Dysfibrinogenemia is relatively frequently found in patients with chronic liver disease.
- Patients with chronic liver disease may develop a consumption coagulopathy—in its most extreme form DIC.
- Recent studies employing sophisticated coagulation tests have shown that due to a rebalancing of the coagulation system in patients with chronic liver failure, thrombin generation is basically normal in the majority of patients, whereas some patients may have a prothrombotic phenotype.

CLINICAL FEATURES

- Patients with liver disease may present with purpura, epistaxis, gingival bleeding, and/or menorrhagia.
- Bleeding typically follow trauma or surgical procedures, especially in sites with high fibrinolytic activity, such as the urogenital tract or oral mucosa.
- Patients with acute viral or toxic hepatitis usually develop abnormal bleeding only if the disease is fulminant.
- Bleeding from esophageal varices requires primary attention to the bleeding site as well as efforts to correct the hemostatic abnormalities.
- The coagulopathy of liver disease may also predispose the patient to thromboembolic complications.

LABORATORY FEATURES

- **Table 83–1** summarizes the laboratory abnormalities that can be found in patients with chronic

liver disease. These abnormalities may both contribute to bleeding or thrombosis.

- Determination of plasma levels of factors V, VII, and VIII may help differentiate liver disease (factor VIII levels normal or increased; factors V and VII decreased), vitamin K deficiency (factor VII decreased; factors V and VIII normal), and DIC (all decreased).

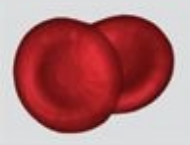
TABLE 83–1 CHANGES IN THE HEMOSTATIC SYSTEM IN PATIENTS WITH LIVER DISEASE THAT CONTRIBUTE TO BLEEDING (LEFT) OR CONTRIBUTE TO THROMBOSIS (RIGHT)

Changes that Impair Hemostasis	Changes that Promote Hemostasis
Primary Hemostasis	
Thrombocytopenia	Elevated levels of VWF
Platelet function defects	Decreased levels of ADAMTS13
Enhanced production of nitric oxide and prostacyclin	
Secondary Hemostasis	
Low levels of factors II, V, VII, IX, X, and XI	Elevated levels of factor VIII
Vitamin K deficiency	Decreased levels of protein C, protein S, antithrombin, α2-macroglobulin, and heparin cofactor II
Dysfibrinogenemia	
Fibrinolysis	
Low levels of α2-antiplasmin, factor XIII, and TAFI	Low levels of plasminogen Increase in PAI-1 levels
Elevated t-PA levels	

ADAMTS13, a disintegrin-like and metalloprotease with thrombospondin domain 13; PAI-1, plasminogen activator inhibitor 1; TAFI, thrombin-activatable fibrinolysis inhibitor; t-PA, tissue-type plasminogen activator; VWF, von Willebrand factor.
Source: *Williams Hematology,* 9th ed, Chap. 128, Table 128–1.

TREATMENT

- Correction of coagulation is only required in case of bleeding or when an invasive procedure has to be performed.
- Replacement of all the deficient coagulation factors may be attempted with fresh-frozen plasma, but large volumes of plasma are required and volume overload may occur. The risk of transmission of infectious agents can be minimized by using solvent-detergent–treated plasma.
- Prothrombin complex concentrates may be used to correct deficiency of the vitamin K–dependent factors but do not contain factor V. These preparations may (theoretically) result in thrombosis and can transmit blood-borne microorganisms.
- Vitamin K administration is effective in patients with vitamin K deficiency. Due to a relative resistance to vitamin K, high doses (10 mg) are advised. Avoid intramuscular injection in coagulopathic patients.
- Platelet transfusion may be useful in correcting thrombocytopenia, but splenic sequestration may reduce the yield to ineffective levels. Trials with thrombopoietin in thrombocytopenic patients requiring invasive procedures are ongoing.
- Antifibrinolytic agents may prevent bleeding in patients with mucosal bleeding or who require dental extraction, but they enhance the risk of thrombosis in patients with DIC.



For a more detailed discussion, see Frank W. G. Leebeeek and Ton Lisman: Hemostatic Dysfunction Related to Liver Diseases and Liver Transplantation, Chap. 128 in *Williams Hematology*, 9th ed.

CHAPTER 84

The Antiphospholipid Syndrome

- The antiphospholipid syndrome is an acquired thrombotic disorder associated with circulating autoantibodies to anionic phospholipid–protein complexes.
- These antibodies were first detected as inhibitors of the partial thromboplastin time in patients with systemic lupus erythematosus (SLE) and, for this reason, were called "lupus anticoagulant," although this finding is not limited to patients with lupus.

PATHOGENESIS

- The disorder is generally considered to be autoimmune, although a direct causal relationship between antiphospholipid antibodies and thrombosis or pregnancy problems has not been demonstrated.
- The antiphospholipid antibodies found in the syndrome usually react with phospholipid bound to a plasma protein.
- A number of pathogenetic mechanisms have been proposed for the antiphospholipid syndrome, and it is possible that several of these act in concert to cause the disorder.

CLINICAL FEATURES

- Patients usually present with manifestations of thrombosis and/or pregnancy complications or loss.
- The disease usually presents in patients between ages 35 and 45 years. Both sexes are equally susceptible.
- The disorder is considered "secondary antiphospholipid syndrome" if the patient has a recognizable autoimmune disease, or "primary antiphospholipid syndrome" if there is no associated disorder.
- **Table 84–1** summarizes the clinical manifestations of the antiphospholipid syndrome.
- The antiphospholipid syndrome should be considered in patients with recurrent thromboses in unusual locations.
- Venous and/or arterial thromboses may occur at any site but are most frequent in the lower extremities.
- Patients with concurrent inherited thrombophilia who develop antiphospholipid antibodies are at increased risk for thrombosis.
- Immune thrombocytopenia, usually of mild to moderate severity, occurs frequently in patients with antiphospholipid syndrome.
- Rarely, patients may develop a *catastrophic* form of the antiphospholipid syndrome, with

severe, widespread vascular occlusions, despite intense anticoagulant treatment, often leading to death.

- Recurrent pregnancy loss occurs often in women with the antiphospholipid syndrome. About one-half of the abortions occur after the first trimester.
- Some patients develop a bleeding disorder because of a concurrent coagulopathy, such as acquired hypoprothrombinemia, or because of acquired inhibitors of factor VIII (see Chap. 82).

TABLE 84–1 CLINICAL MANIFESTATIONS OF THE ANTIPHOSPHOLIPID SYNDROME

- Venous and arterial thromboembolism*
- Pregnancy complications attributable to placental insufficiency, including spontaneous pregnancy losses, intrauterine growth restriction, preeclampsia, preterm labor, and placental abruption*
- Thrombocytopenia
- Thrombotic and embolic stroke*
- Cerebral vein thrombosis*
- Livedo reticularis, necrotizing skin vasculitis
- Coronary artery disease
- Valvular heart disease
- Kidney disease
- Pulmonary hypertension
- Acute respiratory distress syndrome
- Atherosclerosis and peripheral artery disease
- Nonthrombotic retinal disease
- Adrenal failure, hemorrhagic adrenal infarction*
- Budd-Chiari syndrome, mesenteric and portal vein obstructions, hepatic infarction, esophageal necrosis, gastric and colonic ulceration, gallbladder necrosis*
- Catastrophic antiphospholipid syndrome with thrombotic microangiopathy*

*Manifestations that qualify as consensus criteria for diagnosis of antiphospholipid syndrome.
Source: *Williams Hematology,* 9th ed, Chap.131, Table 131–4.

LABORATORY FEATURES

- Diagnosis of the antiphospholipid syndrome requires demonstration of antibodies against phospholipids and/or relevant protein cofactors.
- The most widely recommended tests for antiphospholipid syndrome are anticardiolipin (aCL), anti-β_2 glycoprotein (GP)-I (IgG and IgM), and lupus anticoagulant (LA).
- aCL, IgG and IgM assays are the most sensitive but the least specific. Anti-β_2GPI IgG and IgM assays are more specific but less sensitive.
- Lupus anticoagulant assays, of which the dilute Russell viper venom time is the most common, generally tend to be the least sensitive but the most specific.
- No single test is sufficient for diagnosis, and usually a panel of tests is used.

DIFFERENTIAL DIAGNOSIS

- The diagnosis of antiphospholipid syndrome is based on consensual (research) criteria, as presented in **Table 84–2**.
- Vasculitis may cause vascular occlusion in patients with autoimmune diseases.

- The catastrophic antiphospholipid syndrome should be differentiated from thrombotic microangiopathies (including thrombotic thrombocytopenic purpura) (see Chap. 90), disseminated vasculitis, or disseminated intravascular coagulation (see Chap. 85).
- The "lupus anticoagulant" as the cause of prolongation of the activated partial thromboplastin time should be differentiated from specific coagulation factor deficiencies or other inhibitors by using appropriate laboratory procedures.
- Antiphospholipid antibody levels may be elevated artifactually or because of specific infections, such as syphilis, Lyme disease, HIV, or hepatitis C.

TABLE 84–2 SYDNEY INVESTIGATIONAL CRITERIA FOR DIAGNOSIS OF ANTIPHOSPHOLIPID SYNDROME (APS)

Clinical

- Vascular thrombosis (one or more episodes of arterial, venous, or small vessel thrombosis). For histopathologic diagnosis, there should *not* be evidence of inflammation in the vessel wall.
- Pregnancy morbidities attributable to placental insufficiency, including three or more otherwise unexplained recurrent spontaneous miscarriages, before 10 weeks of gestation. Also, one or more fetal losses after the 10th week of gestation, stillbirth, episode of preeclampsia, preterm labor, placental abruption, intrauterine growth restriction or oligohydramnios that are otherwise unexplained.

Laboratory

- aCL or anti-β_2GPI IgG and/or IgM antibody present in medium or high titer on two or more occasions, at least 12 weeks apart, measured by standard ELISAs.
- Lupus anticoagulant in plasma, on two or more occasions, at least 12 weeks apart detected according to the guidelines of the International Society of Thrombosis and Hemostasis Scientific Standardization Committee on Lupus Anticoagulants and Phospholipid-Dependent Antibodies.
- "Definite APS" is considered to be present if at least one of the clinical criteria and one of the laboratory criteria are met.

aCL, anticardiolipin; aPL, antiphospholipid; β_2 GPI, β_2-glycoprotein I; ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin.

Data from Miyakis S, Lockshin MD, Atsumi T et al: International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* 4:295–306, 2006.

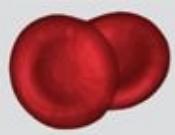
TREATMENT, COURSE, AND PROGNOSIS

Thrombosis

- Acute thrombosis in the antiphospholipid syndrome is treated the same as thrombosis from any cause.
- Patients with the antiphospholipid syndrome who develop spontaneous thromboembolism should receive long-term, possibly lifelong, oral anticoagulation. Clinical studies have not shown conclusive evidence that a higher intensity of anticoagulant therapy should be maintained.
- Hydroxychloroquine therapy appears to have an antithrombotic effect in patients with the antiphospholipid syndrome and SLE.
- Patients with the catastrophic antiphospholipid syndrome may benefit from treatment with anticoagulants, glucocorticoids, and plasma exchange or intravenous gamma-globulin. There is anecdotal successful experience with rituximab.
- Antiphospholipid antibodies may spontaneously disappear and its presence should be monitored.

Pregnancy Loss

- Pregnant patients who have antiphospholipid antibodies but have no history of clinical problems do not require treatment.
- Women who have antiphospholipid antibodies, and who have spontaneously lost three or more pregnancies should receive aspirin and heparin during the pregnancy and after delivery. For example, one suggested regimen calls for treatment with aspirin (80 mg) daily and unfractionated heparin (5000 U subcutaneously every 12 hours) or low-molecular-weight heparin (LMWH) at prophylactic dose beginning with diagnosis of the pregnancy and continuing at least until delivery.
- A recent randomized trial, however, did not show superiority of LMWH plus aspirin over aspirin alone in prevention of miscarriage.
- Patients who have had systemic thromboembolism should be considered for oral anticoagulation for 6 to 12 weeks after delivery. Breastfeeding is possible if the baby is administered usual vitamin K treatment.



For a more detailed discussion, see Jacob H. Rand and Lucia Wolfgast: The Antiphospholipid Syndrome. Chap. 131 in *Williams Hematology,* 9th ed.

CHAPTER 85
Disseminated Intravascular Coagulation

- Disseminated intravascular coagulation (DIC) is a syndrome that is characterized by systemic intravascular activation of coagulation, leading to fibrin deposition in the microvasculature and small- and mid-size vessels, thereby contributing to organ dysfunction. Simultaneously, ongoing consumption of platelets and coagulation factors leads to thrombocytopenia and impaired coagulation and may result in serious bleeding complications.
- DIC never occurs by itself but is always secondary to an underlying cause. **Table 85–1** lists the most frequently occurring disorders know to be associated with DIC.

TABLE 85–1 CLINICAL CONDITIONS THAT MAY BE COMPLICATED BY DISSEMINATED INTRAVASCULAR COAGULATION

Infectious diseases: purpura fulminans
Malignancy
Solid tumors
Leukemias
Trauma
Brain injury
Burns
Liver diseases
Heat stroke
Severe allergic/toxic reactions
Snake bites
Vascular abnormalities/Hemangiomas
Kasabach-Merritt syndrome
Other vascular malformations
Aortic aneurysms
Severe immunologic reactions (eg, transfusion reaction)
Obstetrical conditions
Abruptio placentae
Amniotic fluid embolism
Preeclampsia/eclampsia
HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome
Sepsis during pregnancy
Acute fatty liver

Source: *Williams Hematology,* 9th ed, Chap. 129, Table 129–1.

PATHOGENESIS

- The pathogenesis of DIC is diagrammed in **Figure 85–1**.
- Exposure of blood to tissue factor appears to be the principal mechanism of activation of coagulation. Tissue factor may be expressed by mononuclear cells or by the endothelium.
- Other stimuli include activation of factor Xa by a cancer procoagulant, snake envenomation,

and tissue/cellular debris in patients with massive trauma or pancreatitis.

- Activation of coagulation is insufficiently balanced by physiologic anticoagulant pathways (eg, antithrombin, protein C system) and a downregulation of endogenous fibrinolysis due to high levels of the fibrinolysis inhibitor plasminogen activator inhibitor type 1 (PAI-1).

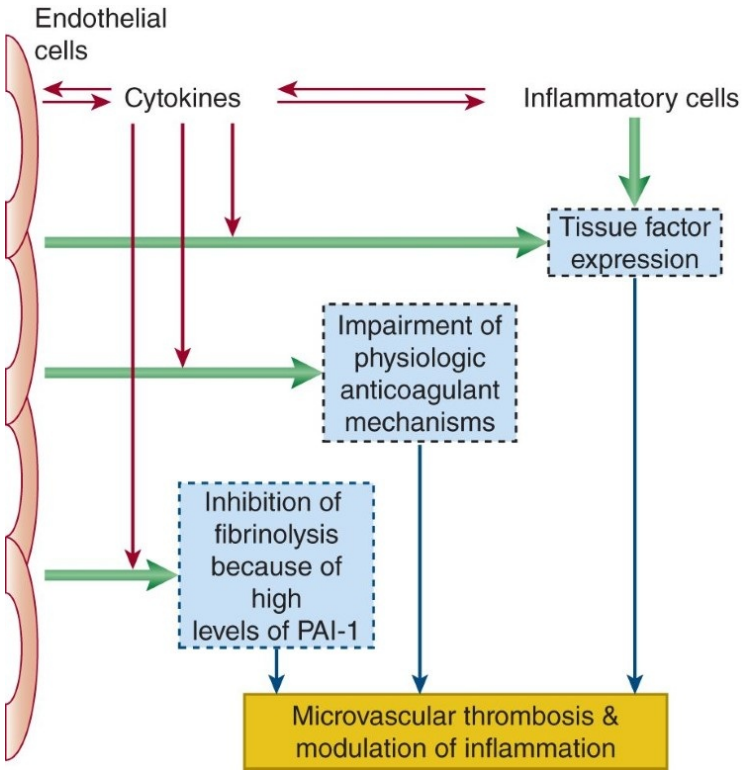


FIGURE 85–1 Schematic presentation of pathogenetic pathways involved in the activation of coagulation in disseminated intravascular coagulation (DIC). In DIC, both perturbed endothelial cells and activated mononuclear cells may produce proinflammatory cytokines that mediate coagulation activation. Activation of coagulation is initiated by tissue factor expression on activated mononuclear cells and endothelial cells. In addition, downregulation of physiologic anticoagulant mechanisms and inhibition of fibrinolysis by endothelial cells further promote intravascular fibrin deposition. PAI-1, plasminogen-activator inhibitor type 1. (Source: *Williams Hematology,* 9th ed, Chap. 129, Fig. 129–1.)

CLINICAL FEATURES

- Clinical features are related to the underlying disorder, to the DIC, or both.
- Bleeding manifestations have been observed in about 25% of cases in several series.
- Persistent bleeding from venipuncture sites or other skin wounds occurs frequently.
- Hemorrhage may be life-threatening.
- Extensive organ dysfunction may be induced by microvascular thrombi, or by venous and/or arterial thromboembolism.
- Organ dysfunction may manifest as acute renal failure (renal cortical ischemia and acute tubular necrosis occur frequently), hepatic dysfunction, and respiratory insufficiency due to acute respiratory distress syndrome.
- Coma, delirium, focal neurologic symptoms, and signs of meningeal irritation may occur because of thrombosis or hemorrhage in the cerebral vasculature.
- Mortality rates range from 30% to 85%. The presence of DIC is a strong predictor for mortality in sepsis, trauma, and other underlying conditions.

LABORATORY FEATURES

- The underlying disorders may influence the abnormalities expected in DIC and must be considered in interpretation of laboratory data.
- There is not a single laboratory test that is able to confirm or reject the diagnosis of DIC.
- Typically, the platelet count is low, prothrombin time (PT) and activated partial thromboplastin time (aPTT) are prolonged, levels of coagulation factors and coagulation inhibitors are low, and fibrin related markers (fibrin degradation products, fibrin monomers, D-dimer) are elevated.
- A simple scoring algorithm, utilizing the platelet count, PT, D-dimer, and fibrinogen level has been proposed by the International Society on Thrombosis and Hemostasis and has been prospectively validated (see **Table 85–2**).
- Fibrinogen levels are rarely low because fibrinogen initially acts as an acute phase protein and levels may markedly increase due to the underlying cause.
- Primary fibrinogenolysis may be distinguished from DIC by finding a normal platelet count, greatly elevated fibrinogen degradation products, and very low levels of α_2-antiplasmin and plasminogen (see Chap. 86).

TABLE 85–2 DIAGNOSTIC ALGORITHM FOR THE DIAGNOSIS OF OVERT DISSEMINATED INTRAVASCULAR COAGULATION (DIC)*

1. Presence of an underlying disorder known to be associated with DIC	❑
(no = 0, yes = 2)	
2. Score global coagulation test results	❑
Platelet count *(> 100 = 0; < 100 = 1; < 50 = 2)*	
Level of fibrin markers (soluble fibrin monomers/fibrin degradation products)	❑
(no increase: 0; moderate increase: 2; strong increase: 3)	
Prolonged prothrombin time	❑
(< 3 s = 0; > 3 s but < 6 s = 1; > 6 s = 2)	
Fibrinogen level	❑
(> 1.0 g/L = 0; < 1.0 g/L = 1)	
3. Calculate score	❑
4. If ≥ 5: compatible with overt DIC; repeat scoring daily	
If < 5: suggestive (not affirmative) for nonovert DIC; repeat next 1–2 days	

*According to the Scientific Standardization Committee of the International Society of Thrombosis and Haemostasis.
Data from Taylor FBJ, Toh CH, Hoots WK, et al. Towards definition, clinical and laboratory criteria, and a scoring system for disseminated intravascular coagulation. *Thromb Haemost.* 2001 Nov;86(5):1327–1330.

TREATMENT

- Rapid and appropriate treatment of the underlying disorder is of utmost importance, including antibiotics and source control for infection, anticancer treatment, surgical and medical management of trauma, or evacuation of a dead fetus.

- Because most patients with DIC are critically ill, appropriate supportive care, including fluids, pressors, dialysis, and respiratory and ventilator management, is essential.
- There is no convincing evidence that transfusion of blood components "fuels the fire," and patients with documented deficiencies who are bleeding or require surgical or invasive procedures should receive transfusion with platelets for thrombocytopenia and fresh-frozen plasma or coagulation factor concentrates for coagulation factor depletion.
- Critically ill patients need prophylaxis for venous thromboembolism and therefore unfractionated or low-molecular-weight heparin is recommended.
- The use of (therapeutic levels of) heparin to ameliorate DIC is a matter of debate. In general, in the absence of adequately controlled studies, there is no sound clinical evidence supporting the use of heparin in DIC.
- Heparin treatment may be beneficial in patients with purpura fulminans (overt hemorrhagic infarction of the skin and underlying tissue), overt thromboembolism, and when thrombosis is likely to cause irreversible tissue injury. In these cases, unfractionated heparin at a dose of 500 to 750 U/h via continuous infusion may be sufficient.
- The decision to use heparin must be individualized, and the risks and benefits considered carefully.
- Administration of recombinant antithrombin (1500–3000 U) or human activated protein C (drotrecogin alfa 24 μg/kg per hour by continuous infusion for 4 days) may ameliorate laboratory parameters in DIC but did not result in an improvement of clinically relevant outcome. These interventions are associated with increased risk of bleeding.
- Antifibrinolytic therapy is generally contraindicated in DIC because it may provoke increased thrombosis and microvascular occlusion but may be considered in patients with severe bleeding when primary fibrin(ogen)olysis, rather than DIC, is the major process (see Chap. 86).

SPECIFIC UNDERLYING DISEASES

Infection

- Neonates, asplenic patients, and pregnant patients are more prone to development of infection-related DIC.
- All microorganisms, including gram-positive and gram-negative bacteria, viruses, parasites, and fungal infections may cause DIC.

Malignancy

- Solid tumors often produce a chronic DIC in which thrombosis is more prominent than bleeding. This syndrome may respond to heparin.
- Patients with acute promyelocytic leukemia (APL) frequently develop major bleeding. The pathogenesis of the hemostatic abnormalities in APL is complex and may involve both DIC and primary fibrin(ogen)olysis. With the use of modern treatment strategies, including all-*trans*-retinoic acid (ATRA), coagulopathy and bleeding has become a less prominent feature of APL (see Chap. 45).
- Acute lymphocytic leukemia has been associated with DIC, particularly with induction

therapy.

Complications of Pregnancy

- Abruptio placentae causes acute DIC because of rapid entry of large quantities of placental tissue factor into the maternal circulation.
- Amniotic fluid embolism is a rare catastrophe that occurs most often in multiparous women undergoing difficult labors with postmature, large fetuses. DIC is caused by entry into the maternal circulation of amniotic fluid that contains tissue factor.
- The dead fetus syndrome occurs several weeks after intrauterine death and is caused by tissue factor from the fetus slowly entering the maternal circulation.
- Rapid volume replacement and evacuation of the uterus are treatments of choice. Replacement therapy with fresh-frozen plasma, coagulation factor concentrates, and platelets is given if severe bleeding occurs.
- The DIC usually rapidly resolves when the underlying cause has been handled properly.
- The syndrome of *h*emolysis, *e*levated *l*iver enzymes, and *l*ow *p*latelets (HELLP) occurs in the third trimester or postpartum. DIC appears to have a role in the pathogenesis of the HELLP syndrome. The HELLP syndrome may be confused with other forms of thrombotic microangiopathy (eg, thrombotic thrombocytopenic purpura–hemolytic uremic syndrome [TTP-HUS]) (see Chap. 90).
- Patients should receive supportive care, careful monitoring, and blood component replacement therapy.

Trauma

- The initial coagulation defect after severe trauma is a dilutional coagulopathy due to blood loss and replacement therapy with red cells and plasma expanders.
- After 24 to 48 hours, a systemic inflammatory response syndrome may occur, leading to frank DIC.
- In the initial phase, restoration of coagulation factors and platelets by fresh frozen plasma and platelet transfusion, respectively, should be initiated. In the later phase, supportive treatment for DIC (see above) should be considered.

Newborns

- Laboratory evidence of DIC in newborns consists of progressive decline in hemostatic parameters; thrombocytopenia; and reduced levels of fibrinogen, factor V, and factor VIII.
- The most frequent underlying causes are sepsis, hyaline membrane disease, asphyxia, necrotizing enterocolitis, intravascular hemolysis, abruptio placentae, and eclampsia.
- Bleeding from multiple sites is the most frequent presentation, but in about 20%, no clinical manifestations of DIC are present.
- Management consists of treatment of the underlying disorder, support of vital functions, and replacement of blood components.

For a more detailed discussion, see Marcel Levi and Uri Seligsohn: Disseminated Intravascular Coagulation, Chap. 129 in *Williams Hematology*, 9th ed.

CHAPTER 86

Fibrinolysis and Thrombolysis

HYPERFIBRINOLYSIS

Pathophysiology

- Local activation of the fibrinolytic system accompanies the formation of the hemostatic plug and is important in repair of injury and reestablishment of blood flow.
- Excessive local or systemic fibrinolysis can prematurely degrade fibrin clots and lead to significant bleeding.

Systemic Hyperfibrin(ogen)olysis

- Endothelial cell plasminogen activator may be released in pathologic states in sufficient amounts to convert plasma plasminogen to plasmin.
- A hemorrhagic state may ensue with the following laboratory features:
  — Shortened euglobulin lysis time
  — Decreased levels of fibrinogen, plasminogen, and α_2-antiplasmin
  — Elevated levels of fibrin(ogen) degradation products
  — Normal platelet count
  — Low levels of factor V and VIII (due to proteolytic degradation by plasmin)
- Localized fibrinolysis may also cause abnormal bleeding in patients with either normal or defective hemostasis.

THROMBOLYTIC TREATMENT

Principles

- All fibrinolytic drugs are enzymes that accelerate the conversion of plasminogen to plasmin, a serine protease that degrades the insoluble fibrin clot matrix into soluble derivatives.
- The basic principle of all fibrinolytic therapy is administration of pharmacologic amounts of plasminogen activator to achieve a high local concentration at the site of the thrombus and thereby accelerate conversion of plasminogen to plasmin and increase the rate of fibrin dissolution.
- If large amounts of plasminogen activator overwhelm the natural regulatory systems, plasmin may be formed in the blood resulting in degradation of susceptible proteins, the “lytic state.” Additionally, because high concentrations of activator are not limited to the site of thrombosis, fibrin deposits at other sites, including physiologic hemostatic plugs needed at sites of injury, may also dissolve causing local bleeding, often exacerbated by the hypocoagulable state caused by proteolysis of other coagulation factors by plasmin.

- Several therapeutic agents are available and approved for thrombolytic use (**Table 86–1**).

TABLE 86–1 COMPARISON OF PLASMINOGEN ACTIVATORS

Agent (Regimen)	Source (Approved)	Antigenic	Half-Life (min)
Streptokinase (infusion)	Streptococcus (Y)	Yes	20
Urokinase (infusion)	Cell culture; recombinant (Y)	No	15
Alteplase (t-PA) (infusion)	Recombinant (Y)	No	5
Anistreplase (bolus)	Streptococcus + plasma product (Y)	No	70
Reteplase (double bolus)	Recombinant (Y)	No	15
Saruplase (scu-PA) (infusion)	Recombinant (N)	No	5
Staphylokinase (infusion)	Recombinant (N)	Yes	
Tenecteplase (bolus)	Recombinant (Y)	No	15

N, no; scu-PA, single chain urokinase-type plasminogen activator; t-PA, tissue-type plasminogen activator; Y, yes.
Source: *Williams Hematology*, 9th ed, Chap. 135, Table 135–3.

Streptokinase

- This single-chain polypeptide derives from β-hemolytic streptococci.
- It lacks intrinsic enzymatic activity but combines stoichiometrically with plasminogen to form a complex that possesses plasmin-like proteolytic activity.
- The streptokinase–plasminogen complex converts free plasminogen to plasmin.
- The activity of streptokinase is enhanced by fibrinogen, fibrin, and fibrin degradation products.
- The streptokinase–plasmin(ogen) complex is itself proteolytically degraded by plasmin.
- Allergic reactions to streptokinase, including fever, hypotension, urticaria, and bronchospasm may occur, necessitating the use of antihistamines and glucocorticoids.
- Neutralizing antibodies are commonly induced after treatment with streptokinase, which abrogates response to further streptokinase therapy at standard doses.

Urokinase

- This serine protease directly activates plasminogen.
- In vivo, it is present in single-chain form (scu-PA) that possesses very low levels of activity and serves as a zymogen, a high-molecular-weight two-chain form (HMW-tcu-PA), and a low-molecular-weight two-chain form (LMW-tcu-PA).

Tissue Plasminogen Activator

- Tissue plasminogen activator (t-PA) is a serine protease synthesized by endothelial cells and commercially available as a recombinant product that activates plasminogen.
- t-PA binds to fibrin, which induces conformational changes in t-PA, in plasminogen, or in both that increase the catalytic efficiency of plasminogen activation several hundred-fold.
- The relative fibrin specificity of t-PA is a theoretical advantage over other fibrinolytic agents but may not be as clinically important as once believed. Effective treatment of arterial

thrombosis entails rapid clot lysis, requiring t-PA doses high enough to provoke a systemic lytic state.
- t-PA does not provoke allergy or antibody formation but is relatively expensive.

Newer Plasminogen Activators

Staphylokinase

- Staphylokinase is a profibrinolytic protein produced by *Staphylococcus aureus*. Its mechanism of action is similar to that of streptokinase.
- This highly efficient fibrinolytic agent produces high rates of clot lysis without significantly changing levels of fibrinogen, plasminogen, or α_2-antiplasmin levels.
- It is effective in preliminary clinical trials on patients with acute myocardial infarction.
- Neutralizing antibodies may develop promptly following therapy.

Mutant Tissue-Type Plasminogen Activators

- Two mutant forms of t-PA, prepared by recombinant technology, t-PAΔ FEK-1 (reteplase) and TNK-t-PA, have been shown in clinical trials to be effective in restoring vessel patency.

CLINICAL USE OF THROMBOLYTIC AGENTS

- Thrombolysis, in particular recombinant tissue-type plasminogen activator, was shown to be effective for ST-elevation acute myocardial infarction. However, when primary percutaneous coronary intervention (with or without stent insertion) is readily available, this is superior to thrombolytic treatment.
- Thrombolysis in acute ischemic stroke is effective, when adhering to strict selection and exclusion criteria (**Table 86–2**). Also here, percutaneous neurovascular intervention (when rapidly available) is more effective.
- Thrombolysis is accepted treatment in patients with severe pulmonary embolism and a compromised hemodynamic state and/or respiratory insufficiency. In less severe pulmonary embolism, thrombolytic treatment is effective but carries a high risk of major bleeding. Hence, in this circumstance, thrombolysis is generally not recommended.
- Thrombolytic treatment may lead to more rapid resolution of deep venous thrombosis and possibly to a lower incidence of postthrombotic venous insufficiency; however, because of the high risk of major bleeding, this treatment is generally not recommended.
- Thrombolytic treatment is often used for local lysis of arterial thrombosis in peripheral arteries, dialysis shunts, or intravenous lines.
- Anecdotal reports document successful treatment of intra-abdominal thrombosis including Budd-Chiari syndrome, portal vein thrombosis, and mesenteric vein thrombosis.

TABLE 86–2 **GUIDELINES FOR TISSUE-TYPE PLASMINOGEN ACTIVATOR THERAPY IN STROKE**

Eligibility
Time from symptom onset to therapy ≤3 h
Results from European Cooperative Acute Stroke Study (ECASS) III trial suggest treatment within 4.5 h of onset is beneficial

Exclusions
Prior intracranial hemorrhage
Major surgery within 14 days
Gastrointestinal or urinary tract bleeding with 21 days
Arterial puncture in noncompressible site
Recent lumbar puncture
Intracranial surgery, serious head trauma, or prior stroke within 3 months
Minor neurologic deficit
Seizure at time of stroke onset
Clinical findings of subarachnoid hemorrhage
Active bleeding
Persistent systolic blood pressure (BP) > 185 mm Hg and/or diastolic BP > 110 mm Hg or requiring aggressive treatment
Arteriovenous malformation or aneurysm
Evidence of hemorrhage on computed tomography scan
Platelets < 100,000/μL
International normalized ratio >1.5 on warfarin
Elevated partial thromboplastin time on heparin
Blood glucose < 40 or > 400 mg/dL
ECASS III additionally excluded patients > 80 years old, patients with a combination of previous stroke and diabetes mellitus, and patients with an National Institutes of Health Stroke Scale score of > 25

Source: *Williams Hematology*, 9th ed, Chap.135, Table 135–6.

THERAPY WITH ANTIFIBRINOLYTIC AGENTS

- **Table 86–3** lists disorders that have been treated with antifibrinolytic therapy.

TABLE 86–3 ANTIFIBRINOLYTIC THERAPY WITH EACA OR TA IN BLEEDING STATES

Pathologic Process and Clinical Bleeding State	Experience Using Antifibrinolytic Agents	Comment
Systemic hyperfibrinolysis		
Inherited α^2-antiplasmin	Controlled with antifibrinolytic agents	Rare autosomal recessive trait; prophylactic treatment indicated only in severe cases
Disseminated intravascular coagulation (DIC)	May aggravate DIC	Treatment indicated only in rare cases with excessive activation of fibrinolysis
Fibrinolytic therapy	Anecdotal	If bleeding is excessive, antifibrinolytic agents may be helpful
Malignancy (solid tumor)	Useful if bleeding is due to hyperfibrinolysis alone (rare)	Hypercoagulable state may be unmasked by antifibrinolytic treatment
Acute promyelocytic leukemia	May reduce bleeding manifestations	Coexistent thrombotic state may preclude use of antifibrinolytic agents
Liver disease and transplantation	Protracted oozing can be better controlled	An excessive hyperfibrinolytic state is present during the anhepatic and immediate postperfusion stages
Extracorporeal bypass surgery	Bleeding reduced	Intrapleural or intrapericardial clots resistant to lysis may occur with treatment
Trauma	Tranexamic acid reduced mortality when administered within 6 h	
Localized fibrinolysis with defective hemostasis		
Hemophilia A and B, von Willebrand disease, and factor XI deficiency	Proven use for dental extractions, probable usefulness after other	Antifibrinolytic agents not effective as prophylaxis for hemarthrosis

	surgical procedures	
Anticoagulated patients	Dental surgery blood loss decreased with administration as mouthwash	
Thrombocytopenia	Controlled trials fail to show benefit	Antifibrinolytic agents may be useful in patients refractory to platelet transfusion
Kasabach-Merritt syndrome	Useful for shrinking hemangioma masses if properly used	Antifibrinolytic agents may trigger systemic DIC
Localized fibrinolysis with normal hemostasis		
Prostatectomy	Reduces postoperative bleeding	Treatment indicated only for cases with severe and prolonged bleeding
Menorrhagia	Effectively reduces blood loss	Evaluate for underlying pathology
Upper gastrointestinal	Useful adjunctive measure to reduce bleeding, studies were done in absence of endoscopic hemostasis	
Subarachnoid hemorrhage	Incidence of rebleeding reduced, but vasospasm is accentuated	No reduction in mortality with treatment
Hereditary hemorrhagic telangiectasia	May reduce severity and frequency of epistaxis and gastrointestinal bleeding	

EACA, ∈-aminocaproic acid; PAI-1, plasminogen activator inhibitor-1; TA, tranexamic acid.
Source: *Williams Hematology*, 8th ed, Chap. 87, Table 87–3.

Synthetic Lysine Analogs

- ∈-Aminocaproic acid and tranexamic acid are synthetic lysine analogs that block the conversion of plasminogen to plasmin by occupying the lysine-binding site on plasminogen that is responsible for binding of plasminogen to fibrin, which accelerates its conversion to plasmin.

∈-Aminocaproic Acid

- Peak plasma levels are achieved by 2 hours after oral dose.
- Eighty percent of intravenous dose is cleared unchanged within 3 hours by the kidney.
- This agent is excreted for 12 to 36 hours because of large volume of distribution.
- It is administered as intravenous priming dose of about 0.1 g/kg body weight over 20 to 30 minutes, followed by continuous intravenous infusion of 0.5 to 1 g/h, or equivalent intermittent dose either intravenously or orally every 1, 2, or 4 hours.
- In patients receiving prolonged treatment with ε-aminocaproic acid, rhabdomyolysis has been described.

Tranexamic Acid

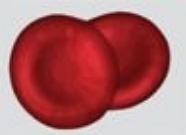
- Plasma half-life is approximately 1 to 2 hours.
- More than 90% is excreted unchanged in urine within 24 hours.
- Oral dosage is 5 to 10 mg/kg three or four times daily. (In practice, it is 500–1000 mg 3–4 times daily.)
- Intravenous dose is 10 mg/kg three or four times daily.
- Infrequently encountered side effects include thrombosis, myonecrosis, or hypersensitivity

reaction.

- Thrombosis risk is most significant when there is an associated thrombogenic process, such as occult disseminated intravascular coagulation.
- In patients with upper urinary tract bleeding, antifibrinolytic therapy can lead to obstructing clots in the urinary collecting system.

Aprotinin

- This polypeptide inhibits serine proteases by forming a 1:1 complex with the enzyme.
- Aprotinin is administered intravenously because of gastric inactivation.
- It is distributed in extracellular space and metabolized by the kidney.
- Potency is expressed as “kallikrein inhibitor units” (KIU), where 10^6 KIU corresponds to 140 mg pure inhibitor.
- Most common side effects are nausea, vomiting, diarrhea, muscle pain, and hypotension.
- Allergic side effects are itching, rash, urticaria, and dyspnea. Cardiovascular collapse, bronchospasm, or anaphylactic shock is rare.
- Because of increased renal complications, cardiovascular morbidity and mortality in patients undergoing cardiac surgery, aprotinin is no longer available in most countries.



For more detailed discussion, see Katherine A. Hajjar and Jian Ruar: Fibrinolysis and thrombolysis, Chap. 135 and Greg C. Connolly and Charles W. Francis: Principles of Antithrombotic Therapy, Chap. 25 in *Williams Hematology*, 9th edition.

PART XI

THROMBOSIS AND ANTITHROMBOTIC THERAPY

CHAPTER 87
Principles of Antithrombotic Therapy

- Antithrombotic agents are characterized separately as anticoagulants (including vitamin K antagonists, heparin or heparin derivatives, and directly acting thrombin or factor Xa inhibitors), antiplatelet agents, or fibrinolytic drugs (see Chap. 86), depending on their primary mechanism, although there is overlap in their activities.
- Anticoagulant therapy acts to decrease fibrin formation by inhibiting the formation and action of thrombin. Its most common use is in preventing systemic embolization in patients with atrial fibrillation, treatment of acute arterial thrombosis (eg, myocardial infarction or peripheral arterial thrombosis) and for treatment or (secondary) prevention of venous thromboembolism.
- Anticoagulant therapy is often monitored using coagulation testing because of marked biologic variation in effect.
- Antiplatelet agents act to inhibit platelet function, and their primary uses are in preventing thrombotic complications of cerebrovascular and coronary artery disease. They also have a role in treatment of acute myocardial infarction. They have no effect in preventing or treating venous thromboembolism.
- For many agents, the risk-to-benefit ratio is narrow, with the result that bleeding complications occur.
- Bleeding is the most common adverse effect of anticoagulation (**Table 87–1**). Consequently, the clinician should carefully weigh the risks and benefits for each patient when selecting treatment.
- The most common oral anticoagulants are vitamin K antagonists (coumarins). However, recently, new oral anticoagulants with specific antithrombin activity or anti–factor Xa activity have become available and are currently evaluated in clinical trials (see section, “Oral Antithrombin and Anti–factor Xa Agents” below).

TABLE 87–1 RISK FACTORS FOR HEMORRHAGIC COMPLICATIONS

Too high intensity of anticoagulation
Simultaneous use of anticoagulants and antiplatelet agents
Old age
Initial phase of anticoagulation
Cerebrovascular disease
History of alcohol abuse
Renal insufficiency
Liver failure
Use of nonsteroidal anti-inflammatory drugs (gastrointestinal bleeding)
Polymorphisms in cytochrome 450 CYP2C9 gene

Source: *Williams Manual of Hematology*, 8th ed, Chap. 88, Table 88–2.

VITAMIN K ANTAGONISTS

- Coumarins act by inhibiting vitamin K–dependent posttranslational γ-carboxylation of glutamic acid residues in the Gla domains of coagulation factors II, VII, IX, and X, and the anticoagulant proteins C and S.
- γ-Carboxylation requires the reduced form of vitamin K as a cofactor. During γ-carboxylation, vitamin K is oxidized. The enzymes vitamin K epoxide reductase and vitamin K reductase are required to recycle vitamin K back to its reduced form. Coumarins inhibit these reductases, thus depleting reduced vitamin K.
- A decrease in the number of γ-carboxyglutamate residues results in coagulation factors with impaired activity because they are unable to bind calcium and undergo necessary conformation changes.
- The production of affected coagulation factors stops promptly, but the anticoagulant effect is delayed until the previously formed coagulation factors are removed from the circulation. Factor VII has the shortest half-life at 6 hours, while the others range from 24 to 72 hours.

Pharmacokinetics

- Warfarin, the most commonly used coumarin, has predictable oral absorption and a half-life of 35 to 45 hours. The pharmacokinetics appear to be dose-dependent.
- It is highly protein-bound and only the free compound is active.
- Warfarin is metabolized by hydroxylation in the liver and excretion of the hydroxylated derivative in the urine. Warfarin is not excreted in significant amounts in breast milk.
- Other frequently used coumarins are phenprocoumon (much longer half-life of 150 to 160 hours) or acenocoumarol (much shorter half-life of 8 to 12 hours).

Administration and Laboratory Monitoring

- Dosages required for adequate anticoagulation range from about 1 to 20 mg per day, probably a result of differences in hydroxylation rates and target-organ sensitivity.
- There is a significant negative correlation between age at start of therapy and dose. Requirement may decrease by 20% over 15 years.
- Warfarin resistance may be caused by impaired absorption, rapid clearance, or decreased affinity of the receptor, but poor compliance, excessive intake of vitamin K, and drug interactions must be ruled out.
- Many drugs interact with vitamin K antagonists, causing either an increased or decreased anticoagulant response (**Table 87–2**). Several mechanisms have been described for these interactions.
- Vitamin K antagonist therapy is monitored by the prothrombin time (PT).
- The sensitivity of the PT to anticoagulation varies with the source of thromboplastin in the assay.
- Interlaboratory variation is corrected for by using the international normalized ratio (INR) instead of the PT ratio.
- The International Sensitivity Index (ISI) is a correction factor established for each thromboplastin. The INR is determined by the formula $INR = (\text{patient PT}/\text{control PT})^{ISI}$.

- A target range of INR 2.0 to 3.0 has shown to be optimal for virtually all indications. Patients with prosthetic heart valves at high risk for thromboembolic complications may benefit from an INR range of 2.5 to 3.5. Also, in some patients with antiphospholipid syndrome and thrombosis, a higher range of 2.5 to 3.5 is recommended.
- In established venous thromboembolism, vitamin K antagonist therapy is given concomitantly with heparin because the antithrombotic effect of vitamin K antagonists is achieved only after 3 to 4 days.
- Some studies have indicated that patients with mechanical heart valves may be effectively treated with a combination of vitamin K antagonists to achieve an INR of less than or equal to 2.5 and an antiplatelet agent, but such regimens carry an increased risk of bleeding complications.
- Bioprosthetic valves also may cause thromboembolism (in particular in the initial phase), and prophylaxis with vitamin K antagonists is recommended to an INR of 2.0 to 3.0 during the first 3 months and continued indefinitely if there is atrial fibrillation, atrial thrombi, or a prior embolism.
- The risk of thromboembolism from cardioversion may be reduced by vitamin K antagonist therapy to an INR of 2.0 to 3.0 for 3 weeks before the procedure and 4 weeks after.

TABLE 87–2 EFFECT OF COMMONLY USED DRUGS ON WARFARIN RESPONSE

Potentiate Effect	
α-Methyldopa	Indomethacin
Acetaminophen	Isoniazid
Acetohexamide	Ketoconazole
Allopurinol	Methimazole
Androgenic and anabolic steroids	Methotrexate
Antibiotics that disrupt intestinal flora (tetracyclines, streptomycin, erythromycin, kanamycin, nalidixic acid, neomycin)	Methylphenidate Nalidixic acid Nortriptyline Oxyphenbutazone
Cephaloridine	*p*-Aminosalicylic acid
Chloral hydrate	Paromomycin
Chloramphenicol	Phenylbutazone
Chlorpromazine	Phenyramidol
Chlorpropamide	Phenytoin
Cimetidine	Propylthiouracil
Clofibrate	Quinidine
Diazoxide	Salicylate
Disulfiram	Sulfinpyrazone
Fluconazole	Sulfonamides
Glucagon	Thyroid hormone
Guanethidine	Tolbutamide
Depress Effect	
Antipyrine	Glutethimide

Azathioprine	Griseofulvin
Barbiturates	Haloperidol
Carbamazepine	Phenobarbital
Digitalis	Prednisone
Ethanol	Rifampin
Ethchlorvynol	Vitamin K

Source: *Williams Hematology,* 9th ed, Chap. 25, Table 25–2.

Adverse Effects and Reversal

Bleeding

- The annual risk of major bleeding episodes has been estimated at between 1.2 and 7.0 per 100 patient-years. The wide variability exists because of differences in intensity of anticoagulation and patient populations and in the definition of "major bleeding."
- The gastrointestinal tract is the most common site of bleeding. Gastrointestinal bleeding in anticoagulated patients may be caused by peptic ulcer or colon cancer. For this reason, detailed investigation to detect the source of bleeding should be carried out.
- Vitamin K antagonist treatment may be reversed by the administration of vitamin K (1–10 mg). However, it will take 6 to 8 hours after intravenous administration and 12 to 14 hours after oral administration of vitamin K before the effect is noticeable.
- Subcutaneous administration of vitamin K is less effective (more variable response) than oral administration. Intramuscular injections of vitamin K should be avoided in anticoagulated patients.
- In patients with major hemorrhage, rapid reversal of anticoagulation can be achieved with replacement therapy using fresh-frozen plasma or prothrombin complex concentrates. It may be difficult to administer a sufficient volume of fresh-frozen plasma to replace the deficient coagulation factors, and therefore prothrombin complex concentrates may be more convenient.
- Reversal of anticoagulant treatment with vitamin K antagonists is only required in case of serious bleeding. A too high INR in the absence of bleeding does not require vitamin K administration (**Table 87–3**) and may make reanticoagulation particularly difficult.
- Minor bleeding (eg, epistaxis) may be managed by local measures if the INR is in the therapeutic range.

TABLE 87–3 REVERSING WARFARIN THERAPY

Indication	Action
INR < 6	Lower the dose, consider withholding 1 or more doses Recheck in 3–7 days
INR 6–10	Lower the dose and withhold 1–3 doses Consider administering vitamin K, 1–2 mg orally Recheck INR in 24–48 h
INR > 10	Withhold doses until INR in desired range and cause of elevation ascertained Give vitamin K, 2–4 mg orally Recheck INR in 24 h
Serious bleeding and major overdose	Administer four-factor prothrombin complex concentrate if available for rapid reversal. If four-factor prothrombin complex concentrate not available administer fresh-frozen plasma. Also

	give 5–10 mg vitamin K intravenously

INR, international normalized ratio.
Source: *Williams Hematology,* 9th ed, Chap. 25, Table 25–4.

Warfarin-Induced Skin Necrosis

- A rare condition in which painful, discolored areas of skin, most often over fatty areas such as the buttocks, breasts, and thighs, appear, usually between the third and tenth day of warfarin therapy.
- Lesions progress to frank necrosis and eschar formation.
- The necrosis appears to be a result of more rapid decline of protein C and protein S levels than levels of factors II, IX, and X, thereby inducing a temporary hypercoagulable state.
- It may occur in patients with heparin-induced thrombocytopenia, in those with hereditary protein C or protein S deficiency, and in patients receiving large loading doses of warfarin.
- Treatment with warfarin should be stopped immediately and the anticoagulation should be reversed by administration of plasma, or administration of protein C concentrate if protein C deficiency is present. Prompt administration of vitamin K may stop the progress of skin necrosis.
- Anticoagulation should be continued with an alternative anticoagulant until healing of the lesions.

Purple Toe Syndrome

- Patients receiving warfarin therapy may develop a syndrome of bilateral burning pain and dark blue discoloration of the toes and sides of the feet. The involved areas blanch with pressure.
- This condition occurs in patients with cardiac disease, diabetes mellitus, or peripheral vascular disease. It may be caused by cholesterol emboli.

Pregnancy

- Vitamin K antagonists are contraindicated in pregnancy because they may induce midface and nasal hypoplasia, stippled epiphysis, hypoplasia of the digits, optic atrophy, and mental impairment in the fetus. These teratogenic effects are mostly associated with use of vitamin K antagonists during the second trimester of pregnancy; however, many believe that vitamin K antagonist should be avoided throughout pregnancy.
- Vitamin K antagonists are contraindicated in the last 4 weeks of pregnancy due to anticoagulation of the child and the risk of intracranial hemorrhage during vaginal delivery.

Perioperative Management of Anticoagulation

- It appears that full anticoagulant therapy can be safely continued with cutaneous surgery, soft-tissue aspirations or injections, and pacemaker surgery.
- Oral surgery is also safe at an INR of less than 2.5, provided adequate local hemostasis and optionally use of tranexamic acid for irrigation at the time of the procedure and as a mouth rinse four times daily for a week postoperatively.
- For all other types of surgery on patients with high risk of thromboembolism, protocols have been developed for temporary discontinuation of vitamin K antagonists and sustained

perioperative anticoagulation with low-molecular-weight heparin (LMWH).
- Spinal or epidural anesthesia as well as local nerve block should be avoided.

HEPARIN AND HEPARIN DERIVATIVES

Mechanism of Action

- Unfractionated heparin consists of a heterogeneous mixture of sulfated glycosaminoglycans of different chain length with an average molecular mass of 15,000 daltons and an average chain length of 50 sugar residues.
- LMWH is prepared by depolymerization of unfractionated heparin by chemical or enzymatic means. The average molecular mass is 4000 to 6000 daltons, with a range of 1000 to 10,000 daltons.
- Heparin enhances the inactivation by antithrombin of thrombin and factors Xa and IXa.
- Inhibition of thrombin by heparin-antithrombin involves formation of a ternary complex, with heparin binding both thrombin and antithrombin.
- Formation of the ternary complex requires a heparin chain of at least 18 saccharide units.
- Inhibition of factor Xa by heparin-antithrombin does not require direct binding of heparin to factor Xa and therefore LMWHs have a relatively high anti–factor Xa over anti–factor IIa activity.
- Synthetic pentasaccharides (eg, fondaparinux) highly selectively bind to antithrombin and have only anti–factor Xa activity.
- Danaparoid is a mixture of glycosaminoglycans, containing heparan sulfate, dermatan sulfate, and chondroitin sulfate. The predominant anticoagulant effect is on factor Xa.
- Danaparoid is used for therapeutic anticoagulation in patients with acute heparin-induced thrombocytopenia or prophylactic anticoagulation in patients with a history of heparin-induced thrombocytopenia.

Pharmacokinetics

- The pharmacokinetics of unfractionated heparin are compatible with saturable binding to endothelial cells and macrophages, combined with unsaturable renal excretion.
- The half-life of heparin increases with increased doses. In general, the half-life of unfractionated heparin at therapeutic dose is approximately 90 minutes.
- Therapeutic doses of unfractionated heparin are commonly administered by continuous intravenous infusion (after a single intravenous loading dose). Prophylactic unfractionated heparin can be given by twice daily subcutaneous injections.
- LMWHs have a more predictable systemic bioavailability after subcutaneous administration and a much longer half-life (12–24 hours). Hence, they are administered by once or twice daily subcutaneous injections, both therapeutically or prophylactically.

Laboratory Monitoring of Therapy

- The activated partial thromboplastin time (aPTT) is the most frequently used test to monitor therapy with unfractionated heparin.
- In patients with venous thromboembolism and acute coronary syndromes, the aPTT response

to a given heparin level is quite variable, and heparin dosages must be adjusted to achieve the desired aPTT range.

- Laboratory monitoring is not required for prophylactic subcutaneous heparin.
- LMWH does generally not require laboratory monitoring. However, in pregnant patients, critically ill patients, and patient with severe renal insufficiency (creatinine clearance < 30 mL/min) measurement of the anti–factor Xa activity in plasma is useful. LMWH cannot be monitored by the aPTT.

Clinical Use

Venous Thromboembolism

- Unfractionated heparin administered at a dose of 5000 units every 8 to 12 hours is widely used for antithrombotic prophylaxis in patients undergoing surgery, in patients with ischemic stroke and leg paralysis, and in general medical patients.
- Alternatively, once daily subcutaneous low-dose LMWH is effective for antithrombotic prophylaxis as well (**Table 87–4**).
- Fondaparinux is more effective and safe compared with LMWH in patients undergoing major orthopedic surgery.
- Randomized clinical trials demonstrate that patients may be effectively treated for venous thromboembolism by heparin given intravenously at an initial loading dose of 5000 units intravenously, followed by maintenance therapy with 750 to 1500 U/h adjusted to the aPTT (aim: 1.5 to 2-fold prolongation of baseline aPTT).
- Venous thromboembolism can also be effectively treated with LMWH or fondaparinux (see **Table 87–4**).
- Adequate initial infusion rates and frequent determination of the aPTT in the first 24 hours reduce the frequency of delayed adequate heparinization. Use of a validated heparin treatment protocol makes it more likely that adequate early heparinization will be achieved.
- Long-term treatment of venous thromboembolism in pregnant patients or for others for whom warfarin is unsatisfactory can be achieved by adjusted-dose subcutaneous heparin.

TABLE 87–4 LOW-MOLECULAR-WEIGHT HEPARIN REGIMENS*

	Drug†	Regimen
Prophylaxis of VTE		
General surgery		
Low risk	Dalteparin	2500 U, 1 or 2 h preoperation and daily
	Enoxaparin	40 mg, 2 h preoperation and daily
	Fondaparinux	2.5 mg daily (start 6–8 h postoperation)
	Nadroparin	2850 anti-Xa U once daily
High risk	Dalteparin	5000 U, 10–14 h preoperation and daily
		2500 U, 1–2 h preoperation and after 12 h; then 5000 U daily (with malignancy)
	Enoxaparin	40 mg, 2 h preoperation and daily
	Fondaparinux	2.5 mg daily (start 6–8 h postoperation)

Orthopedic surgery	Dalteparin	2500 U, 4–8 h postoperation and 5000 U daily; or 2500 U, 2 h preoperation and 2500 U, 4–8 h postoperation and 5000 U daily; or 5000 U, 10–14 preoperation and 5000 U daily
	Enoxaparin	30 mg BID starting 12–24 h postoperation; 40 mg 9–15 h preoperation and once daily
	Fondaparinux	2.5 mg daily (start 6–8 h postoperation)
Medical patients	Enoxaparin	40 mg once daily
	Nadroparin	2850 anti-Xa U once daily
Treatment of VTE	Fondaparinux	weight < 50 kg: 5 mg daily; 50–100 kg: 7.5 mg daily; > 75 kg: 10 mg daily
	Dalteparin (VTE with cancer)	200 U/kg daily × 1 month; then, 150 U/kg daily for up to 6 months
	Enoxaparin	1 mg/kg q12h; 1.5 mg/kg daily
	Tinzaparin	175 U/kg daily
Acute coronary syndrome	Dalteparin	120 U/kg (max 10,000 U) q12h
	Enoxaparin	STEMI: 30 mg IV bolus plus 1mg/kg SQ q12h (older than age 75 y: initial 0.75 mg/kg with no IV bolus)
	Enoxaparin	Unstable angina and non-STEMI: 1 mg/kg 12 h

STEMI, ST-segment elevation myocardial infarction; VTE, venous thromboembolism.
*Consult package insert for more detailed dosing information. Only FDA approved-indications are included.
†Drug brand names: dalteparin, Fragmin; enoxaparin, Lovenox; fondaparinux, Arixtra; tinzaparin, Innohep; nadroparin, Fraxiparine.
Source: *Williams Hematology*, 8th ed, Chap. 23, Table 23–5, p. 358.

Acute Coronary Syndromes

- Heparin therapy is given to patients with acute coronary syndromes to reduce the risk of death, myocardial infarction, mural thrombosis, systemic embolism, and recurrent ischemia (see **Table 87–4**).
- In patients with unstable angina, combined use of intravenous heparin and aspirin is the preferred therapy.
- Low-dose, subcutaneous heparin is widely used in patients with acute myocardial infarction to prevent venous thromboembolism.
- Many patients with acute myocardial infarction receive more intensive heparin therapy either as an adjunct to fibrinolytic therapy or because they are at high risk for mural thrombosis and systemic thromboembolism.
- Patients requiring long-term anticoagulation because of high risk for mural thrombosis and systemic embolism are usually transferred to therapy with vitamin K antagonists.

Side Effects

- The principal side effects of heparin therapy are bleeding and thrombocytopenia.
- Heparin-induced thrombocytopenia is discussed in Chap. 90.
- Thrombocytopenia is less likely to occur with LMWH than with unfractionated heparin. However, LMWH is not recommended for patients who have developed thrombocytopenia while receiving unfractionated heparin.
- Long-term treatment with unfractionated heparin, usually for more than 3 months, may cause

osteoporosis. Clinically significant osteoporosis may occur less frequently with LMWH than with unfractionated heparin.
- Heparin may cause elevation of serum transaminase levels, which return to normal when heparin treatment is discontinued.
- Rare side effects are hypersensitivity; skin reactions, including necrosis; alopecia; and hyperkalemia due to hypoaldosteronism.

Antidote to Heparin

- The anticoagulant effect of unfractionated heparin can be neutralized by intravenous administration of protamine sulfate, which should be considered for use in heparinized patients with major bleeding.
- Dosage is usually calculated assuming 1 mg of protamine sulfate will neutralize 100 units of heparin.
- The maximum recommended dose is 50 mg.
- Heparin is rapidly cleared from the plasma and calculation of the dose of protamine required must consider this important variable.
- LMWH is incompletely neutralized by protamine sulfate, but protamine may still be of benefit in treating bleeding caused by LMWH.

DIRECT THROMBIN AND FACTOR XA INHIBITORS

Hirudin and Derivatives

- Hirudin is a 65–amino acid peptide produced in the salivary gland of the leech. Hirudin is the most potent, naturally occurring, specific inhibitor of thrombin.
- Hirudin directly inactivates thrombin by forming a 1:1 complex.
- Hirudin for clinical use is produced by recombinant DNA technology. Recombinant hirudin is not sulfated on the tyrosine residue and consequently has markedly reduced affinity for thrombin, compared with native hirudin (**Table 87–5**).
- Bivalirudin is a 20–amino acid peptide analog of hirudin that produces transient, albeit potent, inhibition of thrombin.
- Lepirudin is a recombinant form of hirudin approved for use in patients with heparin-induced thrombocytopenia.
- Hirudin has been clinically evaluated in patients with acute coronary syndromes and does not appear to be a major advance. Bivalirudin has been compared with heparin in patients who have angina. It was not more effective than heparin in reducing the cluster outcome of death in the hospital, myocardial infarction, or abrupt vessel closure.
- All hirudin derivatives are cleared by the kidney and have a markedly prolonged half-life in case of renal insufficiency.
- Hirudin derivatives carry a high risk of bleeding and currently there is no antidote available.

TABLE 87–5 CLINICAL INDICATIONS AND USE OF DIRECT THROMBIN INHIBITORS

Agent	Clinical Indication	Regimen	Monitoring
Lepirudin	HIT	0.4 mg/kg bolus, then 0.15 mg/kg/h	aPTT

Bivalirudin	Angioplasty, PCI with HIT	0.75 mg/kg/bolus; then 1.75 mg/kg/h	ACT
Argatroban	HIT	2 μg/kg/min	aPTT
	HIT with PCI	350 μg/kg/min bolus, then 15 to 400 μg/kg/min	ACT

ACT, activated clotting time; aPTT, activated partial thromboplastin time; HIT, heparin-induced thrombocytopenia; PCI, percutaneous coronary intervention.
Source: *Williams Hematology*, 8th ed, Chap. 23, Table 23–6, p. 359.

Argatroban

- Argatroban is a small-molecule arginine derivative that reversibly inhibits thrombin by binding directly to the active catalytic site.
- Argatroban is approved for treatment and prophylaxis of heparin-induced thrombocytopenia and for percutaneous interventions in patients with heparin-induced thrombocytopenia. It also shows some benefit in patients with thrombotic stroke in clinical trials (see **Table 87–5**).
- The anticoagulant effect can be assessed with the aPTT, which correlates well with plasma concentrations of the drug.
- Metabolism is primarily hepatic, and the clearance and half-life are prolonged in patients with hepatic functional abnormalities requiring dose reduction. Renal function has less effect on argatroban pharmacokinetics.
- As with other direct thrombin inhibitors, the main side effect is bleeding, and no specific agent is available to reverse its action.

ORAL ANTITHROMBIN AND ANTI–FACTOR XA AGENTS

- The oral direct-acting antithrombin agent dabigatran was shown to be as effective or superior to vitamin K antagonists in patients with atrial fibrillation and for prevention and treatment of venous thromboembolism.
- The oral direct-acting anti–factor Xa agents—rivaroxaban, apixaban, and edoxaban—were also shown to be as effective or superior to vitamin K antagonists in patients with atrial fibrillation and for prevention and treatment of venous thromboembolism.
- The new oral antithrombin and anti–factor Xa agents were not effective in preventing thromboembolism in patients with prosthetic heart valves.
- Dabigatran, rivaroxaban, apixaban, and edoxaban do not need laboratory monitoring, although data in elderly patients and patients with renal insufficiency are limited. The anti–factor Xa inhibitors can be monitored with the prothrombin time (PT), but not with the INR, and with anti–factor Xa assays. Dabigatran can only be accurately monitored with an ecarin clotting time, which may not be routinely available.
- The anticoagulant effect of dabigatran may be reversed by the administration of a Fab fragment that binds dabigatran (idarucizumab) based on preliminary trial data.
- The anticoagulant effect of the oral factor Xa inhibitors may be reversed by a modified inactive factor Xa molecule (Andexanet). Also, prothrombin complex concentrate may be able to reverse the anticoagulant effect of Xa inhibitors.

ANTIPLATELET DRUGS

- The properties that make platelets useful in the arrest of hemorrhage also allow platelets to form thrombi in vessels, and on heart valves, artificial membranes, and prosthetic devices, in particular in situations with high shear stress.
- Drugs that inhibit platelet function may, therefore, have clinical application in the treatment and prevention of arterial thrombosis (**Table 87–6**).
- Drugs that inhibit platelet function include aspirin, nonsteroidal anti-inflammatory drugs, dipyridamole, thienopyridine derivatives (ticlopidine, clopidogrel and prasugrel), and inhibitors of the platelet glycoprotein (GP) IIb/IIIa receptor.

TABLE 87–6 ANTIPLATELET AGENTS BY MECHANISM OF ACTION AND CLINICAL USE

Agent and Indications		Dosages
Cyclooxygenase inhibitors		
Aspirin	Coronary and cerebrovascular disease	75–650 mg daily
	VTE secondary prevention	
Agents that increase cAMP		
Dipyridamole	Coronary, cerebrovascular, peripheral arterial disease	75–100 mg QID
Pentoxifylline	Peripheral arterial disease	400 mg BID
Cilostazol	Peripheral arterial disease	100 mg BID
ADP receptor blockers		
Ticlopidine	Cerebrovascular disease	250 mg BID
Clopidogrel	Coronary, cerebrovascular disease, PCI	75 mg daily, loading dose 300 mg
Prasugrel	ACS, PCI	10 mg daily, 60-mg loading dose
Ticagrelor	ACS	90 mg BID, 180-mg loading dose
ADP mimetic		
Cangrelor	PCI	30 mcg/kg IV bolus, then 4 mcg/kg/min
$\alpha_{IIb}\beta_3$ inhibitors		
Abciximab	ACS, PCI	0.25 mg/kg, then 10 mcg/kg/min
Eptifibatide	ACS, PCI	ACS 180 mcg/kg, then 2 mcg/kg/min PCI 180 mcg/kg, then 2 mcg/kg/min with 180 mcg/kg at 10 min
Tirofiban	ACS, PCI	0.4 mcg/kg/min × 30 min, then 0.1 mcg/kg/min
Thrombin receptor blocker		
Vorapaxar	Coronary disease, peripheral arterial disease	2.08 mg daily

ACS, acute coronary syndrome; ADP, adenosine diphosphate; cAMP, cyclic adenosine monophosphate; PCI, percutaneous coronary intervention; VTE, venous thromboembolism.
Source: *Williams Hematology*, 9th ed, Chap. 25, Table 25–6.

Aspirin

- Aspirin inhibits prostaglandin synthesis by irreversibly acetylating a critical serine residue in

cyclooxygenase, thereby blocking the formation of thromboxane A_2 (TXA_2). Because platelets cannot synthesize new enzymes, the inhibition is permanent for the life span of the platelet.

- This agent inhibits collagen-induced platelet aggregation and secondary aggregation to weak agonists, such as ADP and epinephrine.
- Effects on aggregation last about 7 days after a single oral dose.
- Inhibition of the synthesis of the potentially antithrombotic prostaglandin, PGI_2 (prostacyclin), occurs in endothelial cells, but the inhibition is short-lived because endothelium can synthesize new enzyme.
- A dose of aspirin that inhibits TxA_2 but not PGI_2 production has not been found, and the optimal dose of aspirin has not been defined for any specific indication.
- The dose used for a specific indication should take into account efficacy, as determined by clinical trials, and adverse effects, which include, most importantly, gastrointestinal bleeding and hemorrhagic stroke.

Nonsteroidal Anti-inflammatory Drugs

- These drugs appear to work by a mechanism similar to aspirin, but as the effect on cyclooxygenase is reversible, the effects are of much shorter duration.

Dipyridamole

- This is a phosphodiesterase inhibitor with vasodilator effects.
- Mechanisms of action may include increasing platelet cyclic AMP levels, or indirectly increasing the plasma levels of adenosine.
- This agent does not inhibit aggregation of platelets in platelet-rich plasma *in vitro*, but does inhibit aggregation of platelets in the presence of erythrocytes, as measured by whole-blood aggregometry.
- Other agents that increase cyclic adenosine monophosphate (cAMP) are pentoxifylline and cilostazol.

Thienopyridine Derivatives (Ticlopidine, Clopidogrel, and Prasugrel)

- These antiplatelet drugs prolong bleeding time and inhibit aggregation induced by ADP and low concentrations of collagen or thrombin.
- Antiplatelet effects are caused by metabolites. The drugs appear to exert their antiplatelet effects by inhibiting the binding of ADP to platelets.
- Drugs are given orally and are fully effective only after 2 to 3 days. Loading doses may accelerate the onset of action.
- The usual dose of clopidogrel is 50 to 100 mg daily.
- Adverse effects include diarrhea and rash. Neutropenia may be severe but is usually reversible. Aplastic anemia and thrombotic thrombocytopenic purpura may occur, in particular with ticlopidine.

Inhibitors of the Platelet GP IIb/IIIa Receptor

- Platelets with absence or blockade of the receptor function of GP IIb/IIIa will not aggregate

with any physiologic agonist.

- Blockade of GP IIb/IIIa can be achieved with monoclonal antibodies or with peptide or nonpeptide agonists.
- Abciximab is a human-mouse chimeric antibody fragment that inhibits platelet aggregation almost completely when 80% of GP IIb/IIIa receptors are blocked and that also inhibits the prothrombinase activity of platelets.
- The platelet count has been reported to be reduced to less than 100×10^9/L in approximately 2% to 6% of patients and to less than 50×10^9/L in 1% to 2%.
- Cyclic peptides (eptifibatide) containing the arginine-glycine-aspartic acid (RGD) sequence or the lysine-glycine-aspartic acid (KGD) sequence bind with high affinity to GP IIb/IIIa and are relatively resistant to enzymatic breakdown.
- Nonpeptide agents (tirofiban) inhibit the binding of adhesive proteins to GP IIb/IIIa, presumably because they mimic the structural features of the RGD sequence.

ANTIPLATELET DRUGS IN CLINICAL MEDICINE

Ischemic Heart Disease

- Aspirin therapy is widely used for both primary and secondary prevention of acute coronary syndromes and other forms of ischemic heart disease.
- Aspirin is also useful alone, or in combination, in treating unstable angina and acute myocardial infarction, and as an adjunct in managing patients after thrombolytic therapy, percutaneous coronary interventions, or coronary artery bypass surgery.
- Thienopyridine derivatives are useful in combination with aspirin in unstable angina and in the prevention of acute occlusion after coronary stenting.
- GP IIb/GP IIa antagonists in combination with other drugs favorably influence unstable angina and evolving myocardial infarction, and prevent ischemic vascular complications following percutaneous coronary interventions.

Valvular Heart Disease

- Oral anticoagulant therapy is generally recommended for patients with prosthetic heart valves, but the addition of aspirin is recommended for patients who have systemic thromboembolism despite adequate anticoagulation.

Cerebrovascular Disease

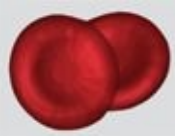
- Antiplatelet therapy is effective in preventing cerebrovascular events in patients with either prior cerebrovascular events or prior cardiac events.
- In most studies, aspirin has been used in doses ranging from 38 to 100 mg/d, but the optimal dose has not been determined. Low doses appear to be as effective as higher doses but have fewer adverse effects.

Peripheral Vascular Disease

- Aspirin treatment may decrease the need for vascular surgery without affecting the pattern of

stable intermittent claudication, suggesting that antiplatelet therapy decreases the incidence of thrombotic complications without affecting the basic disease process.

- The role of antiplatelet therapy in preventing graft occlusion after peripheral artery reconstruction is controversial.



For a more detailed discussion, see Gregory C. Connolly and Charles W. Francis: Principles of Antithrombotic Therapy, Chap. 25 in *Williams Hematology*, 9th ed.

CHAPTER 88
Hereditary Thrombophilia

- Risk factors for thromboembolism may be genetic and acquired (Table 88–1).
- Hereditary thrombophilia is a genetically determined increased risk of thrombosis.
- Up to 50% of patients presenting with a first deep venous thrombosis will have an abnormal laboratory test suggesting a thrombophilic defect, and patients with recurrent thromboses or with a strong family history are even more likely to have laboratory evidence of a thrombophilic state (Table 88–2).
- Up to 16% of patients with thrombophilia have inherited more than one abnormality.
- These inherited defects also interact frequently with acquired risk factors, such as inactivity, trauma, malignancy, or oral contraceptive use, to lead to clinical thrombosis.

TABLE 88–1 THROMBOPHILIAS AND PREDISPOSING RISK FACTORS FOR VENOUS THROMBOEMBOLISM

Thrombophilias	Acquired Predisposing Risk Factors for Venous Thrombosis
Common	Increasing age
Factor V Leiden	Surgery or trauma
Prothrombin G20210A	Prolonged immobilization
Increased factor VIII level*	Obesity
Homozygous C677T polymorphism in methylenetetrahydrofolate reductase†	Smoking
	Malignant neoplasms
Rare	Myeloproliferative diseases
Protein C deficiency	Superficial vein thrombosis
Protein S deficiency	Previous venous thrombosis/varicose veins
Antithrombin deficiency	Pregnancy and puerperium
Very rare	Use of female hormones
Dysfibrinogenemia	Antiphospholipid antibodies/lupus anticoagulants
Homozygous homocystinuria	Hyperhomocysteinemia
	Activated protein C resistance unrelated to factor V Leiden

*Heritability is inferred. No gene alteration has been discerned.

†A questionable thrombophilia that can be associated with hyperhomocysteinemia in patients with deficiencies of folic acid or vitamin B_{12}.

Source: *William Hematology,* 8th ed, Chap. 131, Table 131–1, p. 2122.

TABLE 88–2 FREQUENCY OF THROMBOPHILIAS IN HEALTHY SUBJECTS AND UNSELECTED AND SELECTED PATIENTS WITH VENOUS THROMBOSIS

Type of Thrombophilia	Healthy Subjects (% affected)	Unselected Subjects (% affected)	Selected Subjects (% affected)
Factor V Leiden	5*	19	40
	0.05†		
Prothrombin G20210A	3*	7	16
	0.06†		
Protein C deficiency	0.03	4	5
Protein S deficiency	0.18	2	4
Antithrombin deficiency	0.02	2	4

Data are rounded. Unselected patients are from studies that examined consecutive patients with venous thrombosis. Selected patients are from studies that examined those younger than 50 years of age, with a family history of thrombosis; a history of recurrent events; and absence of acquired risk factors, except for pregnancy and use of oral contraceptives.

*All subjects were of European descent.

†All subjects were of African or Asian descent.

Data from Seligsohn U, Lubetsky A: Genetic susceptibility to venous thrombosis, *N Engl J Med*. 2001 Apr 19;344(16):1222–1231.

HEREDITARY RESISTANCE TO ACTIVATED PROTEIN C

Etiology and Pathogenesis

- Activated protein C (APC) resistance is an abnormally reduced anticoagulant response of a patient's plasma that, in more than 90% of cases, is caused by a genetic abnormality of factor V (substitution of glutamine for arginine at position 506), which significantly retards inactivation of factor Va by APC. The abnormal factor V is generally referred to as "factor V Leiden."

Clinical Features

- The factor V Leiden mutation occurs in 3% to 12% of Caucasians but is rare in other ethnic groups.
- Deep and superficial venous thromboses are the most common manifestations of factor V Leiden, which has been reported to account for 20% to 25% of first thromboembolic events.
- Heterozygosity for factor V Leiden increases the relative risk of developing venous thrombosis four to eight times. However, the vast majority of heterozygous carriers of this mutation will not develop thrombosis during their life. In contrast, it is estimated that one-half of homozygous carriers will have a clinically significant thrombotic episode during their lives.
- The evidence regarding the role of factor V Leiden in recurrent thrombosis is conflicting.
- Factor V Leiden induces a relatively mild hypercoagulable state, but the risks of thrombosis are greatly increased by combination with other inherited disorders, such as antithrombin deficiency, or with acquired risk factors, such as immobility or use of oral contraceptives. Use of contraceptives quadruples the risk of thrombosis in women with factor V Leiden.
- A significantly increased risk of arterial thrombosis has been reported in patients with factor V Leiden and other vascular risk factors, such as smoking.

Laboratory Features

- Patients with APC resistance can be identified by special coagulation assays.
- DNA-based assays provide confirmation for positive coagulation tests and distinguish homozygotes and heterozygotes.

PROTHROMBIN G20210A GENE POLYMORPHISM

Etiology and Pathogenesis

- Substitution of guanylic acid (G) for adenylic acid (A) at nucleotide 20210 in the 3′-untranslated end of the prothrombin gene leads to an elevated plasma prothrombin level and predisposes to thrombosis.

Clinical Features

- This mutation is found primarily in Caucasians.
- The mutation is associated with venous thrombosis in all age groups, sometimes in unusual sites. Arterial thromboses also occur.
- The mutation increases the odds ratio for thrombosis by 2- to 5.5-fold.
- The risk of thrombosis in patients with the G20210A polymorphism is further increased by another inherited thrombophilic state or by other risk factors such as oral contraceptive use or smoking.

Laboratory Features

- Diagnosis depends on DNA analysis to identify the mutation in the prothrombin gene.

HYPERHOMOCYSTEINEMIA

Etiology and Pathogenesis

- Hyperhomocysteinemia is a plasma homocysteine level above the normal range.
- Severe hyperhomocysteinemia, or homocystinuria, is a rare autosomal recessive disorder with neurologic abnormalities, premature cardiovascular disease, stroke, and thromboses.
- Mild to moderate hyperhomocysteinemia is an independent risk factor for arteriosclerosis and arterial thrombosis and for venous thrombosis.
- Homocysteine appears to exert prothrombotic effects by interfering with endothelial cell function.
- Hyperhomocysteinemia may be the result of (1) mutations of enzymes involved in metabolism of sulfur-containing amino acids; or (2) nutritional deficiency of vitamin B_6, vitamin B_{12}, or folic acid; or (3) a combination of these causes.

Clinical Features

- Hyperhomocysteinemia is commonly associated with both venous and arterial thromboses.
- Hyperhomocysteinemia increases the odds ratio for venous thrombosis to 2.5 to 3.0.
- The combination of hyperhomocysteinemia with another prothrombotic disorder, such as factor V Leiden, substantially increases the risk of thromboembolism.

- Hyperhomocysteinemia is a strong predictor of recurrent thrombosis.

Laboratory Features

- Homocysteine levels can be measured on properly collected plasma.
- Mutations in the genes for enzymes concerned with homocysteine metabolism (eg, the *MTHFR* gene) can be determined using molecular biology techniques.

PROTEIN C DEFICIENCY

Etiology and Pathogenesis

- APC functions as an anticoagulant by inactivating activated factor V and activated factor VIII. Deficiency of protein C reduces this anticoagulant effect and leads to hypercoagulability.
- Protein C deficiency is inherited as an autosomal dominant trait.
- Affected heterozygotes have protein C levels of approximately 50%.
- Type I deficiency is caused by decreased synthesis of a normal protein.
- Type II deficiency is caused by production of an abnormally functioning protein.

Clinical Features

- Clinical expression of protein C deficiency is variable, perhaps because of coinheritance of other thrombophilic conditions.
- Most deficient patients are identified by screening apparently normal individuals who have no personal or family history of thrombosis.
- Deep and superficial venous thrombosis is the most common presentation. Venous thrombosis may occur in unusual sites. Arterial thrombosis is uncommon.
- By age 45 years, up to one-half of heterozygous persons from clinically affected families will have had venous thromboembolism.
- Homozygous patients with protein C levels less than 1% may develop severe thrombotic syndromes, such as neonatal purpura fulminans.
- Protein C deficiency may also be responsible for warfarin skin necrosis (see Chap. 90).

Laboratory Features

- Protein C deficiency may be detected by properly performed protein C assays.
- Immunoassays can distinguish type I deficiencies (decreased antigen, decreased activity) from type II (normal antigen, decreased activity).
- The large numbers of mutations thus far identified make DNA analysis impractical.
- In patients who have been treated with warfarin, it is necessary to wait at least 2 weeks after stopping warfarin therapy before measuring protein C levels.

PROTEIN S DEFICIENCY

Etiology and Pathogenesis

- Protein S functions as an anticoagulant by enhancing the activity of APC and also may directly

inhibit factors Va, VIIIa, and Xa.

- Plasma protein S circulates both unbound (free) and bound to C4b-binding protein. Only the free form is active.
- Protein S deficiency is inherited as an autosomal dominant trait.
- Protein S deficiency may be due to reduced synthesis of active protein (type I), normal synthesis of a defective protein (type II), or low levels of free protein S (the active form) combined with normal levels of bound protein S (type III).

Clinical Features

- The clinical features of inherited protein S deficiency are similar to those of protein C deficiency.
- Reduced levels of protein S occur in a number of clinical conditions, including oral contraceptive use, pregnancy, oral anticoagulant therapy, disseminated intravascular coagulation, liver disease, nephrotic syndrome, and inflammatory diseases.

Laboratory Features

- For screening purposes, estimation of free protein S antigen or APC-cofactor anticoagulant activity is better than determining total protein S antigen.
- Assessment of total and free protein S and of protein S activity permits classification into types I, II, and III.
- The high frequency of acquired protein S deficiency makes it difficult to identify hereditary defects.
- DNA techniques may be useful within a family with a previously established mutation, but the large number of mutations otherwise limit their value.

ANTITHROMBIN DEFICIENCY

Etiology and Pathogenesis

- Antithrombin is a protease inhibitor that forms irreversible, inactive complexes with thrombin and factors IXa, Xa, and XIa in reactions that are accelerated by heparin or heparan sulfate on endothelial surfaces.
- Antithrombin deficiency is inherited as an autosomal dominant trait.
- Type I deficiency is a result of reduced synthesis of the antithrombin protein.
- Type II deficiency is a result of production of an antithrombin protein with abnormal function.

Clinical Features

- Venous thrombosis of the lower extremities is the most common presentation. Venous thrombosis may also occur in unusual sites. Arterial thrombosis occurs infrequently.
- Antithrombin deficiency is found in about 1% of individuals younger than 70 years of age with a first documented venous thrombosis.
- The odds ratio for thrombosis in patients with antithrombin deficiency is 10 to 20.
- The occurrence of thrombosis peaks in the second decade of life.
- Coinheritance of another gene for thrombophilia or coexistence of prothrombotic

environmental factors substantially increases the risk of thrombosis.
- Antithrombin deficiency with values less than 5% is extremely rare and causes severe arterial and venous thromboses.
- Resistance to heparin therapy occurs frequently in patients not deficient in antithrombin, and is not a useful indicator of the deficiency.

Laboratory Features

- Antithrombin deficiency can be detected using appropriate functional assays. Immunologic assays are needed to distinguish between type I and type II defects.
- Antithrombin activity levels usually range from 40% to 60% in deficient patients.
- Antithrombin activity may be reduced to similar levels by mild liver disease, thrombosis, or heparin therapy, and it may be necessary to repeat the assays and to perform family studies to establish the diagnosis.

ELEVATED LEVELS OF FACTOR VIII AND OTHER COAGULATION FACTORS

- Factor VIII levels above 150% of normal have been defined as an independent risk factor for thrombosis.
- Preliminary data suggest that elevation of levels of factors V, IX, X, and XI above 150% similarly predispose to thrombosis.
- The mechanism of elevation of coagulation factor levels is unknown. Pathogenesis of the thrombi may be increased thrombin generation.
- The clinical features of patients with elevated factor VIII levels are those of patients with other forms of thrombophilia.
- Levels of factor VIII antigen are increased corresponding to factor VIII procoagulation activity.

HEREDITARY THROMBOTIC DYSFIBRINOGENEMIA

- Dysfibrinogenemia is a qualitative defect in the molecule that can be asymptomatic (50%), or lead to either bleeding (30%) or thrombosis (20%). See Chapter 80.
- Dysfibrinogenemia is found in approximately 0.8% of patients presenting with thromboembolism.
- Patients with thrombotic dysfibrinogenemia usually present with venous thrombosis in the third to fourth decade of life.
- These patients have an increased rate of spontaneous abortion and stillbirth and may have postpartum hemorrhage.
- Prolongation of a dilute thrombin time or a reptilase time, and a disparity between levels of immunoreactive and clottable fibrinogen are common in dysfibrinogenemia.

OTHER POTENTIAL THROMBOPHILIC DISORDERS

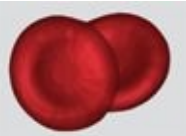
- Hereditary defects of the fibrinolytic system or of thrombomodulin are potential causes of thrombophilia but are not yet clearly established.

DIAGNOSIS OF THROMBOPHILIA

- There is increasing consensus that routine testing for thrombophilia in patients with venous thromboembolism in routine practice is not useful because of a complete lack of clinical consequences and the associated costs.
- Nevertheless, when the choice for testing is made, comprehensive analysis of patients with venous thromboembolism should include the assays for the common and rare causes of thrombophilia as listed in **Table 88–1**.
- Thrombophilic factors can be evaluated in patients receiving oral anticoagulants, except for protein C resistance, and protein C and protein S levels. Proteins C and S can be assayed in blood from patients who have received heparin therapy instead of oral anticoagulants for approximately 2 weeks before performing the tests. Factor V Leiden genotype can be performed instead of testing for APC resistance.
- Women with prior thromboembolism or with a strong family history of thromboembolism may be evaluated for thrombophilia before oral contraceptives are administered.
- Children with venous or arterial thrombosis are likely to have thrombophilia.
- Diagnostic studies for thrombophilia should be considered for women with recurrent midtrimester fetal loss or other adverse pregnancy outcomes.

TREATMENT OF THROMBOPHILIA

- Patients with thrombophilia who develop thrombosis or pulmonary embolism should be treated according to standard protocols for treatment of venous thromboembolism—that is, they should initially receive standard treatment with heparin followed by vitamin K antagonists to maintain the INR between 2 and 3. There is no need for prolonged treatment after a first episode of venous thromboembolism.
- After a recurrent thromboembolic event in a patient with thrombophilia, prolonged anticoagulant treatment is advised, sometimes for indefinite duration.
- If oral anticoagulant therapy is not continued, antithrombotic prophylaxis with low-molecular-weight heparin can be initiated with high-risk events such as surgery, trauma, an intercurrent infection, or immobility.
- B vitamins and folic acid are known to reduce plasma homocysteine levels, but their preventive value is not established. In clinical practice, however, this treatment is often prescribed.
- Low-molecular-weight heparin therapy should be considered for pregnant women who have had previous thromboembolism. The optimal dose of heparin in this situation is subject of ongoing clinical trials.
- Venous thromboembolism that occurs during pregnancy requires heparin throughout the pregnancy and anticoagulant therapy for 4 to 6 weeks postpartum (see Chap. 87).



For a more detailed discussion, see Saskia Middeldorp and Michiel Coppens: Hereditary Thrombophilia, Chap. 130 in *Williams Hematology*, 9th ed.

CHAPTER 89

Venous Thromboembolism

- Venous thromboembolism (deep venous thrombosis and/or pulmonary embolism) is a common disorder, which is estimated to affect 900,000 patients each year in the United States.
- Pulmonary embolism may cause sudden or abrupt death, underscoring the importance of prevention as the critical strategy for reducing death from pulmonary embolism.
- Of the estimated 600,000 cases of nonfatal venous thromboembolism in the United States each year, approximately 60% present clinically as deep venous thrombosis and 40% present as pulmonary embolism.
- Most clinically important pulmonary emboli arise from proximal deep venous thrombosis (thrombosis involving the popliteal, femoral, or iliac veins). Upper extremity deep venous thrombosis also may lead to clinically important pulmonary embolism. Other less common sources of pulmonary embolism include the deep pelvic veins, renal veins, inferior vena cava, right side of the heart, and axillary veins.
- Acquired and inherited risk factors for venous thromboembolism have been identified (for inherited thrombophilia, see Chap. 88). The risk of thromboembolism increases when more than one predisposing factor is present.

CLINICAL FEATURES

- The clinical features of deep venous thrombosis and pulmonary embolism are nonspecific.

Venous Thrombosis

- The clinical features of venous thrombosis include leg pain, tenderness, and asymmetrical swelling, a palpable cord representing a thrombosed vessel, discoloration, venous distention, prominence of the superficial veins, and cyanosis.
- In exceptional cases, patients may present with phlegmasia cerulea dolens (occlusion of the whole venous circulation, extreme swelling of the leg, and compromised arterial flow).
- In 50% to 85% of patients, the clinical suspicion of deep venous thrombosis is not confirmed by objective testing. Conversely, patients with florid pain and swelling, suggesting extensive deep venous thrombosis, may have negative results by objective testing. Patients with minor symptoms and signs may have extensive deep venous thrombi.
- Although the clinical diagnosis is nonspecific, prospective studies have established that patients can be categorized as low, moderate, or high probability for deep venous thrombosis using clinical prediction rules that incorporates signs, symptoms, and risk factors.

Pulmonary Embolism

- The clinical features of acute pulmonary embolism include the following symptoms and signs that may overlap:
  — Transient dyspnea and tachypnea in the absence of other clinical features
  — Pleuritic chest pain, cough, hemoptysis, pleural effusion, and pulmonary infiltrates noted on chest radiogram caused by pulmonary infarction or congestive atelectasis (also known as *ischemic pneumonitis* or *incomplete infarction*)
  — Severe dyspnea and tachypnea and right-sided heart failure
  — Cardiovascular collapse with hypotension, syncope, and coma (usually associated with massive pulmonary embolism)
  — Several less common and nonspecific clinical presentations, including unexplained tachycardia or arrhythmia, resistant cardiac failure, wheezing, cough, fever, anxiety/apprehension, and confusion
- All of these clinical features are nonspecific and can be caused by a variety of cardiorespiratory disorders.
- Patients can be assigned to categories of pretest probability using implicit clinical judgment, or clinical decision rules.

DIAGNOSIS

- Objective diagnostic testing is required to confirm or exclude the presence of venous thromboembolism.
- An appropriately validated assay for plasma fibrin degradation product D-dimer, if available, provides a simple, rapid, and cost-effective first-line exclusion test in patients with low, unlikely, or intermediate clinical probability.
- Integrated diagnostic strategies for deep venous thrombosis and pulmonary embolism are presented in **Figure 89–1** and **Figure 89–2**, respectively.

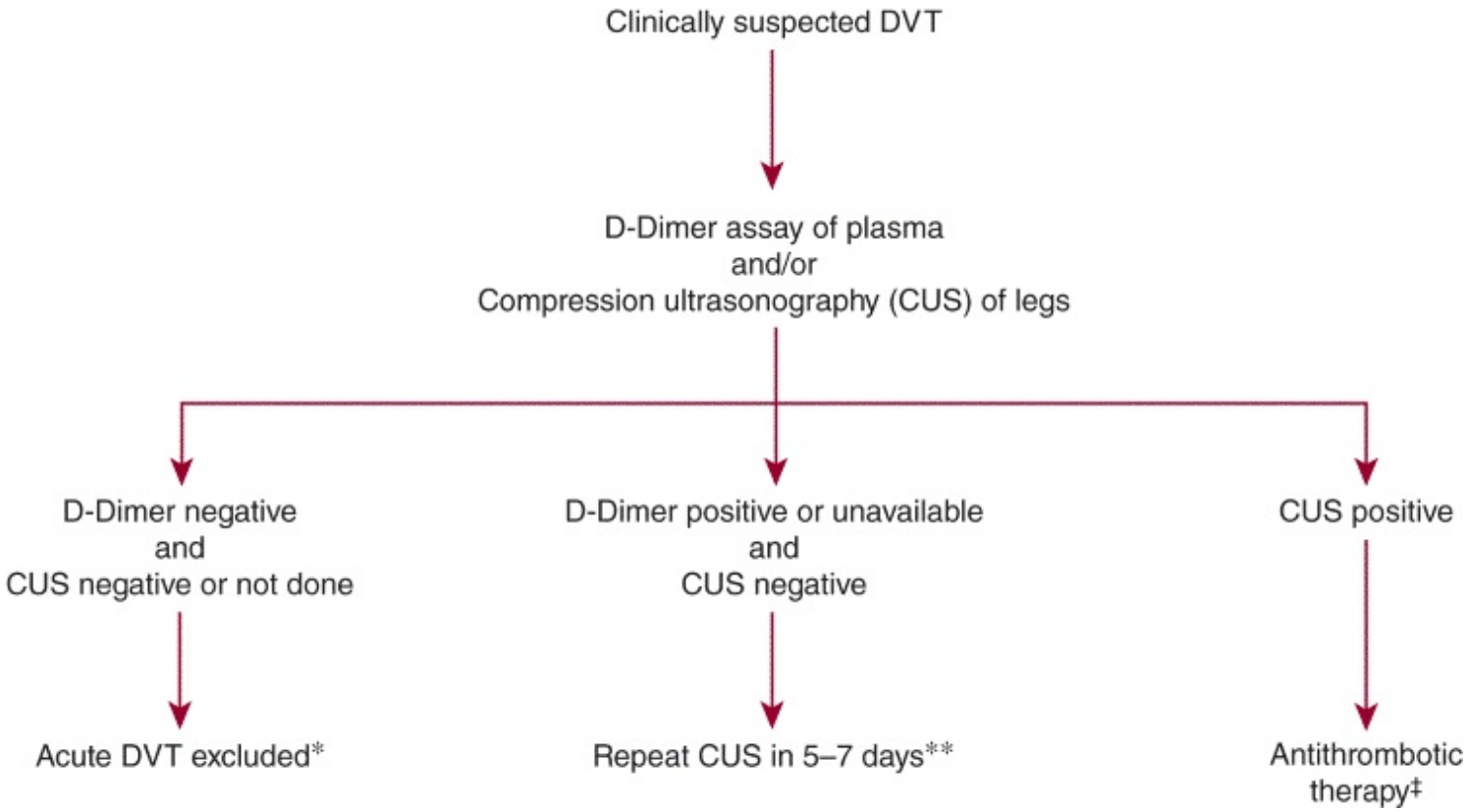


FIGURE 89–1 Diagnosis of patients with suspected first episode of deep venous thrombosis (DVT). *Negative D-dimer can be used to exclude acute DVT, without the need for further diagnostic testing with compression ultrasonography (CUS), if the patient has low, unlikely, moderate, or intermediate clinical probability. Ultrasonography should be performed in patients with a high clinical probability. A negative D-dimer can also be used with a negative CUS at presentation to exclude acute DVT without the need for a repeat CUS. **CUS is performed with imaging of the common femoral vein in the groin and of the popliteal vein in the popliteal fossa extending distally 10 cm from midpatella. A repeat CUS is required in 5 to 7 days to detect extending calf vein thrombi. In centers with the expertise, a single negative result of full-leg duplex ultrasonography (CUS plus flow evaluation) is sufficient to exclude acute DVT. ‡CUS that indicates noncompressibility of deep vein segments is highly predictive of DVT (> 95%) and provides an indication for antithrombotic therapy in most patients. If CUS is positive at a single site isolated in the groin, additional testing with venography, computed tomography, or magnetic resonance imaging should be performed because of the potential for false-positive CUS results from disorders producing vein compression in the groin (eg, tumor mass). (Source: *William Hematology*, 9th ed, Chap. 133, Fig. 133–1.)

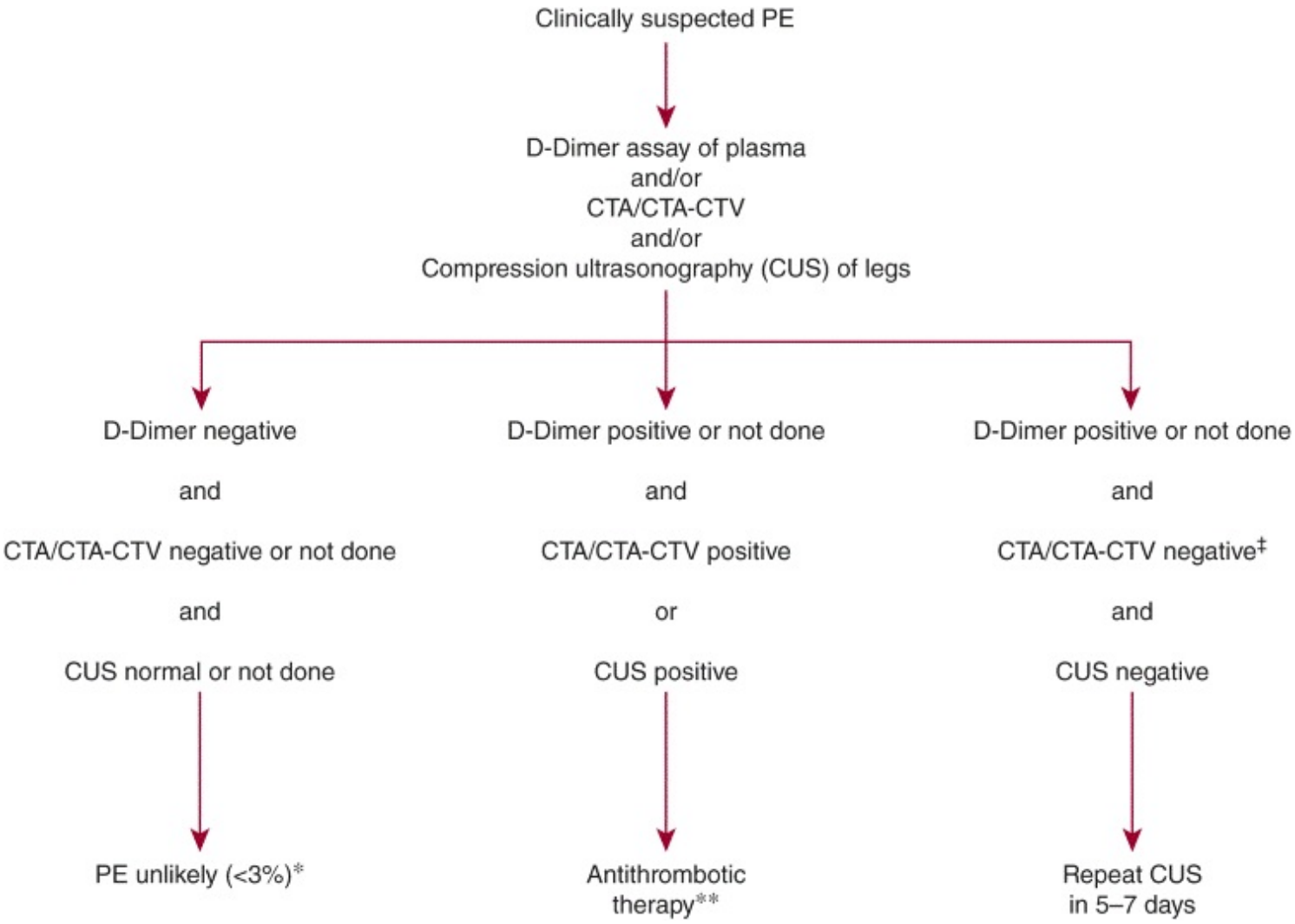


FIGURE 89–2 Integrated strategy for diagnosis of patients with suspected pulmonary embolism (PE) using computed tomographic angiography (CTA) as the primary imaging test. *Negative D-dimer alone can be used as an exclusion test with high negative predictive value (> 97%) in patients with low or moderate probability by the clinical assessment. Patients with a high clinical probability should undergo imaging with CTA or combined CTA-CT venography (CTV). **Positive results on CTA or combined CTA-CTV, in patients with a high or moderate probability of PE by the clinical assessment, have positive predictive value of 90% or more for venous thromboembolism. Similarly, abnormal results by compression ultrasonography (CUS) of the proximal deep veins of the legs have high positive predictive value for proximal vein thrombosis and provide an indication to give antithrombotic therapy. If the patient has a low probability by the clinical assessment, positive results by CTA or CTA-CTV in the main or lobar pulmonary arteries are still highly predictive (97%) for the presence of PE; further testing is recommended for patients with low clinical probability and positive CTA results only of segmental or subsegmental arteries, and the options include pulmonary arteriography or serial CUS. ‡Negative results by CTA or by combined CTA-CTV have high negative predictive value (96%) in patients with low probability by the clinical assessment. For patients with moderate clinical probability, the negative predictive value for combined CTA-CTV is also high (92%), but slightly lower for CTA alone (89%); in this latter group, and in patients with a high probability by the clinical assessment, serial CUS or pulmonary arteriography are recommended options. (Source: *William Hematology*, 9th ed, Chap. 133, Table 133–2.)

Venous Thrombosis

- Enzyme-linked immunosorbent assay (ELISA) and quantitative rapid ELISA for D-dimer have high sensitivity (96%) and negative likelihood ratios of approximately 0.10 for deep venous thrombosis in symptomatic patients.
- Compression ultrasonography of the proximal veins performed at presentation can safely exclude clinically important deep venous thrombosis in symptomatic patients (with the exception of pelvic thrombosis, which may be missed by ultrasound examination).

- The positive predictive value of a positive ultrasonographic result isolated to the calf veins may vary among centers based on expertise and thrombosis prevalence. To detect calf vein thrombi that were initially missed but may have progressed to proximal venous thrombosis, ultrasonography is repeated after 5 to 7 days.
- In centers with the expertise, a single comprehensive evaluation of the proximal and calf veins with duplex (Doppler) ultrasonography is sufficient.
- Measurement of D-dimer using an appropriate assay method can be combined with ultrasonograph imaging of the leg veins. If the two tests are negative at presentation, repeat ultrasonograph imaging is unnecessary.

Pulmonary Embolism

- If capability for combined computed tomographic angiography (CTA) and computed tomographic venography (CTV) exists, it is the preferred approach for most patients with suspected pulmonary embolism because it provides a definitive basis to give or withhold antithrombotic therapy in more than 90% of patients.
- CTA is not inferior to using ventilation–perfusion lung scanning for excluding the diagnosis of pulmonary embolism when either test is used together with venous ultrasonography of the legs.
- Single-detector spiral CT is highly sensitive for large emboli (segmental or larger arteries) but is much less sensitive for emboli in subsegmental pulmonary arteries.
- Multidetector row CT, together with the use of contrast enhancement, has further improved the utility of CT for the diagnosis of pulmonary embolism, also in subsegmental pulmonary arteries.
- Contrast-enhanced CTA has the advantage of providing clear results (positive or negative), good characterization of nonvascular structures for alternate or associated diagnoses, and the ability to simultaneously evaluate the deep venous system of the legs (CTV).
- Ventilation–perfusion lung scanning is another imaging option for the diagnosis of pulmonary embolism. A normal perfusion lung scan excludes the diagnosis of clinically important pulmonary embolism.
- A high-probability lung scan result (ie, large perfusion defects with ventilation mismatch) has a positive predictive value for pulmonary embolism of 85% and provides a diagnostic endpoint to give antithrombotic treatment in most patients.
- The major limitation of lung scanning is that the results are inconclusive in most patients, even when considered together with the pretest clinical probability. The nondiagnostic lung scan patterns are found in approximately 70% of patients with suspected pulmonary embolism.
- Magnetic resonance imaging appears to be highly sensitive for pulmonary embolism and is a promising diagnostic approach. However, clinically important interobserver variation exists in the sensitivity for pulmonary embolism, ranging from 70% to 100%.
- Pulmonary angiography using selective catheterization of the pulmonary arteries is a relatively safe technique for patients who do not have pulmonary hypertension or cardiac failure. If the expertise is available, pulmonary angiography should be used when other approaches are inconclusive and when definitive knowledge about the presence or absence of pulmonary embolism is required.
- Objective testing for nonsymptomatic deep venous thrombosis is useful in patients with suspected pulmonary embolism, particularly those with nondiagnostic lung scan results or

inconclusive CT results. Detection of proximal venous thrombosis by objective testing provides an indication for anticoagulant treatment, regardless of the presence or absence of pulmonary embolism, and prevents the need for further testing.

LONG-TERM SEQUELAE OF VENOUS THROMBOEMBOLISM

- The postthrombotic syndrome is a frequent complication of deep venous thrombosis.
- Symptoms of the postthrombotic syndrome are pain, heaviness, swelling, cramps, and itching or tingling of the affected leg. Ulceration may occur. Symptoms usually are aggravated by standing or walking and improve with rest and elevation of the leg.
- Application of a properly fitted graded compression stocking, as soon after diagnosis as the patient's symptoms will allow and continued for at least 2 years, is effective in reducing the incidence of postthrombotic symptoms, including moderate-to-severe symptoms.
- Chronic thromboembolic pulmonary hypertension is a serious complication of pulmonary embolism and may occur in 1% to 3% of patients.
- Chronic thromboembolic pulmonary hypertension may be suspected if clinical signs and symptoms of pulmonary embolism persist over months despite adequate treatment and can be confirmed by echocardiography and ventilation–perfusion lung scanning.

TREATMENT

- The objectives of treatment in patients with established venous thromboembolism are to:
  — Prevent death from pulmonary embolism
  — Prevent morbidity from recurrent venous thrombosis or pulmonary embolism
  — Prevent or minimize the post-thrombotic syndrome
- Antithrombotic treatment is highly effective for venous thromboembolism. The principles of antithrombotic treatment are outlined in Chapter 87.
- Treatment is initiated with unfractionated or low-molecular-weight-heparin (LMWH) or a heparin derivative for 5 to 10 days (see **Table 89–1**). Alternatively, in case of anticoagulation with rivaroxaban or apixaban, the initiation phase with heparin can be omitted.
- Long-term antithrombotic treatment is currently achieved by administration of vitamin K antagonists (eg, warfarin) or direct oral anticoagulant agents (dabigatran, rivaroxaban, apixaban, or edoxaban).
- The appropriate duration of oral anticoagulant treatment for venous thromboembolism is at least 3 months in patients with a first episode of proximal venous thrombosis or pulmonary embolism secondary to a transient or reversible risk factor.
- Patients with a first episode of idiopathic (unprovoked) venous thromboembolism should be treated for at least 6 months.
- The decision on the duration of antithrombotic treatment should be individualized, taking into consideration the estimated risk of recurrent venous thromboembolism, risk of bleeding, and patient compliance and preference.
- Patients with a first episode of venous thrombosis and a single thrombophilic risk factor (eg, factor V Leiden) do not need prolonged antithrombotic treatment (see Chap. 88).

- Prolonged or even indefinite therapy is recommended for patients with recurrent thrombosis and/or persistent strong risk factors (eg, active cancer or antiphospholipid antibodies) in whom risk factors for bleeding are absent and in whom good anticoagulant control can be achieved. If indefinite anticoagulant treatment is given, the risk-to-benefit ratio of continuing such treatment should be reassessed at periodic intervals.
- Long-term treatment with subcutaneous LMWH for 3 to 6 months is at least as effective as, and in cancer patients is more effective than, oral vitamin K antagonists. However, the repeated subcutaneous injections are not always well tolerated by patients.
- Insertion of an inferior vena cava filter is indicated for patients with acute venous thromboembolism and an absolute contraindication to anticoagulant therapy.
- In patients with a temporary absolute contraindication to anticoagulant treatment (ie, intercurrent bleeding or the need to undergo an invasive procedure), a retrievable inferior vena cava filter is preferable.
- The use of a permanent vena cava filter results in an increased incidence of recurrent deep venous thrombosis 1 to 2 years after insertion (increase in cumulative incidence at 2 years increases from 12% to 21%). If a permanent filter is placed, long-term anticoagulant treatment should be given as soon as safely possible to prevent morbidity from recurrent deep venous thrombosis.

TABLE 89–1 ANTICOAGULANT DRUG REGIMENS FOR TREATMENT OF VENOUS THROMBOEMBOLISM

Drug	Regimen
Low-molecular-weight heparins	
Enoxaparin	1.0 mg/kg BID*
Dalteparin	200 IU/kg once daily†
Tinzaparin	175 IU/kg once daily‡
Nadroparin	6150 IU BID if patient weighs 50–70 kg 4100 IU BID if patient weighs < 50 kg 9200 IU BID if patient weighs > 70 kg
Reviparin	4200 IU BID if patient weighs 46–60 kg 3500 IU BID if patient weighs 35–45 kg 6300 IU BID if patient weighs > 60 kg
Indirect factor Xa inhibitor	
Fondaparinux	7.5 mg once daily if patient weighs 50–100 kg 5.0 mg once daily if patient weighs < 50 kg 10.0 mg once daily if patient weighs > 100 kg
Direct oral anticoagulants	
Dabigatran	150 mg BID after 5 days of parenteral low-molecular-weight heparin or heparin
Rivaroxaban	15 mg BID for 21 days, then 20 mg once daily Taken with food
Apixaban	10 mg BID for 7 days, then 5 mg BID After 6 months, 2.5 mg BID for extended therapy
Edoxaban	60 mg once daily after 5 days of parenteral low-molecular-weight heparin or heparin§

*A once-daily regimen of 1.5 mg/kg can be used but probably is less effective in patients with cancer.

†After 1 month, can be followed by 150 IU/kg once daily as an alternative to an oral vitamin K antagonist for long-term treatment.

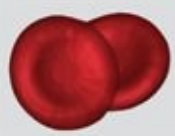
‡This regimen can also be used for long-term treatment as an alternative to an oral vitamin K antagonist.

§30 mg once daily if patient's creatinine clearance is 30–50 mL/min or weight is ≤ 60 kg or if patient is taking strong P-glycoprotein inhibitor drugs.

Source: *William Hematology,* 9th ed, Chap. 133, Table 133–2.

Venous Thromboembolism in Pregnancy

- Adjusted-dose subcutaneous heparin is an appropriate long-term anticoagulant regimen for pregnant patients with venous thromboembolism (see also Chap. 87).
- LMWH does not cross the placenta, and initial experience suggests these agents are safe for treatment of venous thromboembolism in pregnant patients. With regard to safety advantages, LMWH causes less thrombocytopenia and potentially less osteoporosis than unfractionated heparin.
- An additional advantage is that LMWH is effective when given once daily, whereas unfractionated heparin requires twice-daily injection.
- Therapeutic LMWH in pregnancy should be monitored regularly with measurement of plasma anti–factor Xa activity.
- After delivery antithrombotic treatment may be switched to vitamin K antagonists. Breastfeeding while using vitamin K antagonists is possible, provided that the baby receives the usual vitamin K administration that is common in breastfed infants.



For more detailed discussion, see Gary E. Raskob, Russel Hull, and Harry R. Buller: Venous Thrombosis, Chap. 133 in *Williams Hematology*, 9th ed.

CHAPTER 90

Antibody-Mediated Thrombotic Disorders: Thrombotic Thrombocytopenic Purpura and Heparin-Induced Thrombocytopenia

- Thrombotic microangiopathies are characterized by thrombocytopenia, microangiopathic hemolytic anemia, and microvascular thrombosis, leading to variable injury of the central nervous system, kidney, and other organs.
- The classic form of thrombotic microangiopathy (ie, thrombotic thrombocytopenic purpura [TTP]) is usually associated with an acquired (autoimmune) deficiency of ADAMTS13, a metalloprotease that cleaves the ultralarge multimers of von Willebrand factor normally produced by endothelial cells but that are hypercoagulable.
- Hemolytic uremic syndrome (HUS) refers to the thrombotic microangiopathy that mainly affects the kidney and may be diarrhea-associated (caused by enteric infection with Shiga-toxin producing gram-negative microorganisms) or atypical, often due to abnormalities in the regulation of the complement cascade.
- Secondary thrombotic microangiopathies occur in association with infections, certain drugs, metastatic cancer, malignant hypertension, or after stem cell transplantation.
- Heparin-induced thrombocytopenia (HIT) is a significant complication of heparin treatment, associated with mild to moderate thrombocytopenia and a high frequency of both arterial and venous thrombosis. HIT is caused by the formation of anti–heparin/platelet factor-4 antibodies that activate platelets, leukocytes, and endothelial cells.

THROMBOTIC THROMBOCYTOPENIC PURPURA

Etiology and Pathogenesis

- Most cases of TTP are caused by autoantibodies that inhibit ADAMTS13. Congenital deficiency of ADAMTS13 (Upshaw-Schulman syndrome) is rare but well established (see Chap. 88).
- The underlying mechanism causing TTP is unregulated von Willebrand factor–dependent platelet thrombosis.
- Ultra-large von Willebrand factor multimers are released from endothelial cells of the vessel wall and mediate platelet adhesion by binding connective tissue at sites of vascular injury and to platelet glycoprotein Ib on the platelet surface. ADAMTS13 cleaves the von Willebrand factor multimers, thereby preventing platelet-vessel wall interactions in the absence of vascular injury.
- Deficiency of ADAMTS13 leads to spontaneous microvascular platelet thrombosis, causing microvascular obstruction and microangiopathic hemolytic anemia.

Epidemiology and Clinical Features

- The incidence of TTP in the United States has been estimated at approximately 4.5 per million per year.
- The peak incidence is between ages 30 and 50 years, and the disease is rare before age 20 years. The female-to-male ratio averages approximately 2:1, but female preponderance is even more pronounced at relatively younger ages.
- Other risk factors for TTP include African ancestry and obesity and genetic risk factors, such as a low frequency of HLA-DR53.
- The onset of TTP can be dramatically acute or insidious, developing over weeks.
- Approximately one-third of patients have symptoms of hemolytic anemia. Thrombocytopenia typically causes petechiae or purpura; oral, gastrointestinal or genitourinary bleeding is less common but can be severe.
- Systemic microvascular thrombosis can affect any organ, and the consequences are variable. Renal involvement is common, but acute renal failure occurs in fewer than 10% of cases. Neurologic findings can be transient or persistent and may include headache, visual disturbances, vertigo, personality change, confusion, lethargy, syncope, coma, seizures, aphasia, hemiparesis, and other focal sensory or motor deficits.
- Many patients have fever. The symptoms of TTP sometimes can be quite atypical, either at first presentation or on relapse. Thrombocytopenia without hemolytic anemia may herald the onset of disease. In rare instances, visual disturbances, pancreatitis, stroke, or other thrombosis may precede overt thrombotic microangiopathy by days to months.
- Cardiac involvement may cause chest pain, myocardial infarction, congestive heart failure, or arrhythmias. Direct pulmonary involvement is uncommon but severe acute respiratory distress syndrome may occur, possibly secondary to cardiac failure.
- Gastrointestinal symptoms are common and can include abdominal pain, nausea, vomiting, and diarrhea. Physical examination may suggest acute pancreatitis or mesenteric ischemia.

Laboratory Features

- Because the symptoms and signs of TTP are nonspecific, the diagnosis depends on laboratory testing to document microangiopathic hemolytic anemia and thrombocytopenia, without another predisposing cause.
- Thrombocytopenia typically is severe, and one-half of patients have platelet counts below approximately 20×10^9/L. Signs of hemolysis, especially schistocytes, are frequently present although the direct antiglobulin (Coombs) test is almost always negative.
- The hemoglobin level is variable but can be quite severely reduced, and it is best characterized as a microangiopathic hemolytic anemia with schistocytes (fragmented red cells, which are occasionally absent), varying degrees of reticulocytosis, hypohaptoglobinemia, and a negative direct antiglobulin (Coombs) test.
- Almost all patients have normal values for prothrombin time (PT) and activated partial thromboplastin time (aPTT), reflecting only a very minor role of intravascular coagulation in TTP. Mildly elevated fibrin degradation products have been reported in some patients.
- Severe congenital ADAMTS13 deficiency (level < 5%) is characteristic of congenital TTP. Severe acquired ADAMTS13 deficiency appears to be specific for TTP, although the sensitivity of the association is debated and the frequency of severe ADAMTS13 deficiency in

TTP depends on how patients are ascertained.

Differential Diagnosis

- Many diseases associated with secondary thrombotic microangiopathy can produce overlapping clinical and laboratory findings. As a consequence, making a diagnosis of TTP can be a challenge, and a wide differential diagnosis often must be considered (**Table 90–1**).
- Schistocytes occur in a variety of conditions besides TTP, although the level seldom enters the 1% to 18% range typical of TTP. Severe Coombs-negative hemolysis and marked schistocytosis sometimes occur in patients with defective mechanical heart valves.
- Conditions resulting in disseminated intravascular coagulation sometimes cause microangiopathic changes and thrombocytopenia with little change in blood coagulation tests, which can suggest a diagnosis of TTP. Infections may trigger disease in patients with severe ADAMTS13 deficiency, but more commonly, infections cause secondary thrombotic microangiopathy by other mechanisms.
- Recipients of solid-organ transplantations can develop thrombotic microangiopathy, often dominated by renal involvement associated with immunosuppression by cyclosporine or tacrolimus. These drugs appear to damage renal endothelial cells directly and can cause neurotoxicity, adding another feature suggestive of TTP.
- Similarly, hematopoietic stem cell transplantation recipients may develop thrombotic microangiopathy associated with high-dose chemotherapy or radiation, immunosuppressive drugs, graft-versus-host disease, or infections.
- Thrombotic microangiopathy occurs in a small fraction of patients with almost any cancer but most commonly with adenocarcinoma of the pancreas, lung, prostate, stomach, colon, ovary, breast, or unknown primary site. In most cases, the cancer is widely metastatic.
- The differential diagnosis of thrombotic microangiopathy in pregnancy includes preeclampsia, eclampsia, HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome, acute fatty liver of pregnancy, abruptio placenta, amniotic fluid embolism, and retained products of conception. In addition, pregnancy can sometimes trigger disease in patients with congenital or acquired ADAMTS13 deficiency; in most case series of TTP, between 12% and 31% of patients are pregnant women, usually in the third trimester or immediately postpartum.
- Autoimmune thrombocytopenia may be confused with TTP if other causes of microangiopathic hemolytic anemia are present. Asymptomatic thrombocytopenia also may sometimes be the only finding in TTP, as demonstrated by a previous or subsequent episode of disease. Patients have been described in whom TTP and autoimmune thrombocytopenia appeared to occur simultaneously or sequentially. Evans syndrome (autoimmune hemolytic anemia with autoimmune thrombocytopenia) usually can be distinguished from TTP by a positive Coombs test and the prominence of spherocytes relative to schistocytes in the blood film. HIT may sometimes resemble TTP, with thrombocytopenia and disseminated arterial and venous thrombosis (see “Heparin-Induced Thrombocytopenia,” below).
- Systemic lupus erythematosus (SLE) can cause autoimmune hemolysis and thrombocytopenia, and lupus vasculitis can cause microangiopathic changes, renal insufficiency, and neurologic defects consistent with TTP. Vasculitis associated with other autoimmune disorders can pose a similar diagnostic problem.
- Thrombotic microangiopathy can develop in patients with antiphospholipid syndrome (APS),

with or without concurrent SLE (see Chap. 84). The clinical features resemble HUS, catastrophic APS, malignant hypertension, TTP, or HELLP syndrome. One-third of patients present during pregnancy or in the postpartum period.

- Among the drugs that have been associated with thrombotic microangiopathy, the antiplatelet drugs ticlopidine and clopidogrel are unusual because they appear to induce autoantibody inhibitors of ADAMTS13, effectively causing TTP. Thrombotic microangiopathy occurs in 200 to 625 per 1 million users of ticlopidine, usually between 2 and 12 weeks after starting therapy. The incidence of TTP with clopidogrel is lower and is estimated to be 10 per 1 million users. A comprehensive list of other drugs causing TTP can be found in **Table 90–2**. Drugs commonly implicated include selected antineoplastic agents, cyclosporine A, tacrolimus, and quinine.
- Malignant hypertension, which is associated with microangiopathic hemolytic anemia, thrombocytopenia, neurologic symptoms, and renal insufficiency, therefore may resemble TTP.

TABLE 90–1 CLASSIFICATION AND DIFFERENTIAL DIAGNOSIS OF THROMBOTIC MICROANGIOPATHY

Thrombotic Thrombocytopenic Purpura (TTP)
Autoimmune, with antibodies against ADAMTS13
Congenital Thrombotic Thrombocytopenic Purpura (Upshaw-Schulman Syndrome)
Inherited ADAMTS13 deficiency, with mutations in *ADAMTS13*
***Shiga* Toxin-Producing *Escherichia Coli* Hemolytic Uremic Syndrome (STEC-HUS)**
Atypical Hemolytic Uremic Syndrome (aHUS)
Alternative complement pathway defects
Diacylglycerol kinase ε (DGKE) defects
Secondary Thrombotic Microangiopathy
Disseminated intravascular coagulation
Infections (viral, bacterial, fungal)
Streptococcus pneumoniae
Tissue transplant-associated
Chemotherapy or radiation injury
Tissue rejection
Graft-versus-host disease
Cancer
Pregnancy associated (preeclampsia, eclampsia, HELLP [hemolysis, elevated liver enzymes, low platelet count] syndrome)
Autoimmune disorders
Systemic lupus erythematosus and other vasculitides
Antiphospholipid syndrome
Drugs (commonly implicated)
Immune (quinine, ticlopidine)
Toxic (cyclosporine, tacrolimus, mitomycin C, gemcitabine)
Cobalamin metabolic defects
Malignant hypertension
Mechanical hemolysis (eg, malfunctioning aortic or mitral valve prosthesis)

Source: *Williams Hematology*, 9th ed, Chap.132, Table 132–1.

TABLE 90–2 DRUGS AND TOXINS ASSOCIATED WITH SECONDARY MICROANGIOPATHY

Immune-mediated:	**Hormones:**
Quinine	Estrogen/progestogen oral contraceptives
Ticlopidine	Mestranol, norethindrone
Clopidogrel	17β-Estradiol transdermal patch

Antineoplastic agents:
All-*trans* retinoic acid
Bleomycin plus cisplatin
Carmustine
Chlorozotocin
Cytosine arabinoside
Daunorubicin
Deoxycoformycin
Estramustine
Gemcitabine
Lomustine (CCNU)
Mitomycin C
Tamoxifen (when combined with mitomycin C)

Antiangiogenic agents:
Bevacizumab
Sunitinib

Immunosuppressive and anti-inflammatory agents:
Cyclosporine
Tacrolimus
Penicillamine
Muromonab-CD3 (OKT3)
Interferon-α
Interferon-β
Ibuprofen

Antibiotics:
Ciprofloxacin
Clarithromycin
Cephalosporin
Piperacillin
Rifampicin
Metronidazole
Pentostatin
Sulfonamides
Penicillin
Ampicillin
Oxophenarsine
Valacyclovir
Famciclovir
Mefloquine
Conjugated estrogens

Illicit drugs:
Cocaine
Heroin
Ecstasy

Lipid-lowering agents:
Atorvastatin
Simvastatin

H_2-receptor antagonists:
Cimetidine
Famotidine

Vaccinations:
Polio vaccination
Measles/mumps/rubella vaccination
Bacillus Calmette-Guerin (intravesicular)
Influenza vaccination

Miscellaneous:
Bee sting
Bupropion
Chlorpropamide
Procainamide
Iodine
Carbon monoxide
Chloronaphthalene (in varnish)
Aminocaproic acid
Echinacea extract
Quetiapine

HEMOLYTIC UREMIC SYNDROME

- Diarrhea-associated HUS can occur at any age but affects mainly children younger than 10 years.
- The disease occurs sporadically and in epidemics, associated with ingestion of foods or other materials contaminated with Shiga toxin–producing bacteria. *Escherichia coli* O157:H7 accounts for at least 80% of cases in many series, but HUS can also be caused by other toxin-bearing *E. coli* serotypes or by *Shigella dysenteriae* type 1.
- Within 3 days of ingesting the bacteria, patients develop painful diarrhea, without fever, that usually evolves to bloody diarrhea within a few days. HUS may develop during the subsequent 2 weeks, with the acute onset of microangiopathic hemolytic anemia, thrombocytopenia, and

renal injury. ADAMTS13 levels are normal in diarrhea-associated HUS.

- Diarrhea-negative HUS or atypical HUS is much less common than diarrhea-associated HUS. At least half of cases appear to be caused by inherited defects in complement regulatory proteins and activating components, including mutations in complement factor H, factor I, factor H-related proteins 1 and 3 (CFHR1, CFHR3), and C4 binding protein (C4BP). In addition, autoantibodies to factor H have been identified in some patients with atypical HUS, often in association with mutations in CFHR1 and CFHR3.
- The clinical presentation of diarrhea-negative HUS may be sporadic, recessive, or dominant. Many patients develop HUS in childhood, but some have their first episode in adulthood or remain asymptomatic. Occasional patients have long intervals between exacerbations, which may appear to be precipitated by infections, other illness, or pregnancy. The disease often recurs in patients with transplanted kidneys, probably because kidney transplantation does not alter the underlying complement defect.

TREATMENT OF THROMBOTIC MICROANGIOPATHIES

- The mainstay of therapy for TTP is plasma exchange (see **Table 90–3**), which removes antibody inhibitors and replenishes ADAMTS13.
- With the exception of factor H deficiency, and possibly APS and quinine-induced disease, no compelling evidence indicates that plasma therapy is effective for thrombotic microangiopathy caused by a mechanism other than ADAMTS13 deficiency. Regardless of mechanism, however, the clinical features are variable and overlapping. Consequently, plasma exchange may sometimes be used to treat apparent HUS or secondary thrombotic microangiopathy, particularly in adults, based on the possibility that such patients may have an atypical presentation of TTP that will respond.
- After diagnosing TTP, or determining that the diagnosis is sufficiently likely to justify treatment, plasma exchange therapy should be started immediately. The optimal dose of plasma is not known, but a common practice is to perform plasma exchange once daily at a volume of 40 or 60 mL/kg, equivalent to 1 or 1.5 plasma volumes.
- Prompt treatment is essential and if plasma exchange must be delayed more than a few hours, plasma should be given by simple infusion at 20 to 40 mL/kg total dose per day, consistent with the patient's ability to tolerate the fluid load.
- Plasma exchange should be continued daily until the patient has a complete response, as shown by a platelet count greater than 150×10^9/L, lactic acid dehydrogenase (LDH) within the normal range, and resolution of nonfocal neurologic symptoms.
- The optimal schedule for tapering and discontinuing therapy has not been determined. A typical (however, not evidence-based) strategy is to continue plasma exchange until a complete response (normal LDH and platelet count) is sustained for a minimum of 2 days, and then reduce the frequency of plasma exchange to every other day (or twice per week) for several days. If the disease remains quiescent, then treatment can be stopped and the patient monitored closely for recurrence.
- The long-term mortality of TTP treated with plasma exchange ranges from 10% to 20%. Most deaths occur within a few days after presentation, and almost all occur within the first month.

- The duration of illness is quite variable. Complete response occurs after an average of 9 to 16 days of plasma exchange, and almost all responders are encompassed by a range of 2 to 40 days.
- Recurrences of disease more than 30 days after a complete response, occur in up to one-third of patients. Most relapses occur during the first year, but they have been documented up to 13 years after diagnosis.
- TTP often is an autoimmune disease and the use of glucocorticoids is logical, although a beneficial effect has not been demonstrated conclusively. Common practice is to give prednisone or equivalent at a total daily dose of 1 or 2 mg/kg, in one or two doses, for the duration of plasma exchange, followed by tapering. An alternative regimen is methylprednisolone 1 g intravenously daily for 3 days. Rituximab is also being tested to reduce the rate of relapse.
- The use of antiplatelet agents in TTP is controversial. Aspirin and dipyridamole often are combined with plasma exchange but have not been shown conclusively to modify the course of TTP. Low-dose aspirin (eg, 80 mg/d) has been suggested for thromboprophylaxis, once the platelet count exceeds 50×10^9/L.
- Transfusion of platelets may sometimes correlate with the acute deterioration and death in TTP, although direct harm is difficult to establish. Consequently, platelet transfusions are relatively contraindicated and should be reserved for the treatment of life-threatening hemorrhage, preferably after plasma exchange treatment has been initiated.
- TTP that is refractory to plasma exchange may respond to immunosuppression.
- Rituximab (375 mg/m^2 weekly for two to eight doses) resulted in an approximately 95% remission in patients with therapy-resistant TTP within 1 to 3 weeks of starting treatment, including a normal ADAMTS13 level and disappearance of anti–ADAMTS13 antibodies (if present).
- Anecdotal experience suggests that vincristine may be beneficial, although its efficacy is difficult to assess. Dosing schedules have included 2 mg intravenously on day 1 followed by 1 mg on days 4 and 7, or 2 mg intravenously per week for 2 to 14 weeks. Cyclosporine has been used to treat TTP and may be effective in refractory disease. Apparent responses, with normalization of ADAMTS13 activity, have been observed with cyclosporine 2 to 3 mg/kg daily in two divided doses as an adjunct to plasma exchange, or without plasma exchange for early recurrences of TTP.
- Other immunosuppressive regimens have included oral or intravenous cyclophosphamide; oral azathioprine; combination chemotherapy with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP); and autologous stem cell transplantation.
- Many reports suggest that splenectomy can result in lasting remissions or reduce the frequency of relapses for some patients with TTP who are refractory to plasma exchange or immunosuppressive therapy, presumably by removing a major site of anti–ADAMTS13 antibody production.
- The cornerstone for treatment of secondary thrombotic microangiopathy is management of the underlying disorder. In most cases, this is sufficient to ameliorate the manifestations of the thrombotic microangiopathy.

TABLE 90–3 **AN APPROACH TO THE TREATMENT AND MONITORING OF THROMBOTIC**

THROMBOCYTOPENIC PURPURA
Treatment: Glucocorticoids (eg, prednisone 2 mg/kg per day or equivalent) Plasma exchange 1.5 volumes per day Plasma infusion 15–30 mL/kg if plasma exchange will be delayed >12 hours After the platelet count exceeds 50 x 10^9/L, add aspirin 80 mg/day (optional) and routine thromboprophylaxis (eg, low-molecular-weight heparin) Continue until complete response for 3 days (platelets >150 × 10^9/L, LDH normal), then decrease plasma exchange to every other day for two more treatments and stop If response is durable, taper glucocorticoids
Monitoring: Neurologic status Hemoglobin and platelet count Blood film for schistocytes LDH Serum electrolytes, calcium, BUN, creatinine Electrocardiogram, cardiac enzymes
Common complications: Cardiac arrhythmias, infarction Catheter-associated bleeding or thrombosis Citrate toxicity (hypocalcemia, alkalosis) Minor allergic reactions to plasma

BUN, blood urea nitrogen; LDH, lactic acid dehydrogenase; TTP, thrombotic thrombocytopenic purpura.

HEPARIN-INDUCED THROMBOCYTOPENIA

Epidemiology

- The frequency of HIT depends on the nature of the heparin used, dose and duration of heparin exposure, and clinical setting.
- The frequency of HIT in nonsurgical settings is clearly higher in patients treated with unfractionated heparin (1%–5%) than in patients treated with low-molecular-weight heparin (0.2%–1%). Bovine-derived heparin may be associated with a higher incidence of HIT than porcine heparin. Newer, synthetic pentasaccharide anticoagulants may have a much lower or no risk of inducing HIT.
- HIT may be prevented by limiting the exposure time to heparin and avoiding heparin flushes through intravenous lines. Heparin-bonded catheters can cause HIT.
- The greatest clinical risk factors for developing HIT are the patient's age and the nature of the patient's medical condition. HIT occurs rarely or never in pediatric patients, especially neonates. Patients being treated for medical conditions have a lower risk of developing HIT than do patients who are undergoing surgical procedures. Among surgical patients, those undergoing coronary artery bypass grafting, orthopedic procedures, or isolated limb perfusion are particularly vulnerable to developing HIT. Of note, dialysis patients almost never have HIT, despite very regular heparin administration.
- Determination of the incidence of thrombosis in various settings has been hampered by the infrequency of HIT and the need to carefully document both the diagnosis and the thrombotic complications. Nevertheless, some prospective studies suggest that the incidence of thrombosis is between 35% and 58% in patients with documented HIT. The ratio of arterial to

venous thrombi is high (0.7:1).

Etiology and Pathogenesis

- HIT is an immune complex disorder of heparin therapy involving heparin–platelet factor (PF)-4 complexes. Such antibodies are not demonstrable in other forms of thrombocytopenia.
- Binding of these antibodies to platelets lead to platelet activation through the FcγRIIA and the formation of procoagulant platelet microparticles that contribute to the thrombosis.
- As part of this activation, HIT antibodies also bind to endothelial cells likely via PF4-surface glycosaminoglycan complexes. This binding may further increase vascular activation, augmenting local thrombosis.

Clinical Features

- The diagnosis of HIT is difficult to establish in a complicated patient who can have multiple causes for developing thrombocytopenia or thrombosis. A scoring system based on the severity of thrombocytopenia, timing of onset of symptoms, occurrence of thrombosis, and potential thrombocytopenia from other causes ("4 Ts") was developed to help maintain focus on potentially affected patients (**Table 90–4**).
- Typically, patients develop thrombocytopenia 5 to 10 days after the onset of heparin therapy unless exposure occurred within the preceding 3 months.
- Bleeding manifestations secondary to the thrombocytopenia, such as petechiae, nosebleeds, and oozing from catheter sites, are not seen in HIT.
- Symptoms of venous thrombosis include those related to deep venous thrombosis of the lower or upper extremity and pulmonary embolism, adrenal infarctions, and cerebral venous thrombosis. Major venous obstruction can lead to limb gangrene.
- Arterial thrombi in this disease can be striking and were the first feature that led to the recognition of HIT as a distinct clinical entity. Common thrombotic complications include stroke, myocardial infarction, bowel infarction from mesenteric artery thrombosis, and renal infarction.

TABLE 90–4 THE 4 TS

	Points Per Category		
Clinical Sign	**0**	**1**	**2**
Thrombocytopenia (acute)	Very low nadir (10×10^9/L) or < 30% fall	Low nadir ($10–20 \times 10^9$/L) or 30%–50% fall	Moderate nadir (20–100 $\times 10^9$/L) or > 50% fall
Timing of 1st event (thrombocytopenia or thrombosis)	≤ 4 days (unless prior heparin exposure in last 3 months)	Within 5–10 days (but not well documented) or ≤ 1 day (with exposure in last 3 months)	Documented occurrence in 5–10 days or ≤ 1 day with recent prior exposure
Thrombotic-related event	None	Common thrombi (DVT or line thrombus) or recurrent thrombus; erythematous skin lesion or not suspected thrombus	Major vessel thrombus or skin necrosis or skin lesion at site of heparin infusion
Thrombocytopenia (other causes)	Definite other cause is present	Possible other cause is present	No other strong explanation for thrombocytopenia

A score of 6–8 is high risk for HIT, 4–5 is intermediate risk, and 0–3 is low risk.
DVT, deep venous thrombosis.
Data from Reilly MP, Taylor SM, Hartman NK, et al: Heparin-induced thrombocytopenia/thrombosis in a transgenic mouse model requires human platelet factor 4 and platelet activation through FcgammaRIIA, *Blood* 2001 Oct 15;98(8):2442–2447.

Laboratory Features

- Thrombocytopenia is the key laboratory finding in HIT. Most often it is moderate, ranging from 20 to 100 × 10^9/L, or is represented by a 50% to 70% decline in platelet count. Rarely is the thrombocytopenia more severe or absent, as observed in some cases with skin necrosis.
- Although thrombin generation increases in HIT, patients rarely have decompensated disseminated intravascular coagulation.
- Two assay prototypes for confirming the diagnosis of HIT are available. One measures the Ig antibodies to the heparin–PF4 complex (antigen assay), and the other measures heparin-dependent antibodies that activate platelets (activation assay) in plasma or sera.
- Antigen assays are easily established in the laboratory and have a rapid turnaround time. The specificity of this test for the diagnosis of HIT is, however, limited. A recently described cause for a false-positive antigen test is the presence of anti-PF4 rather than anti-PF4/heparin antibodies.
- The activation assays are not commercially established because specific platelet donors are needed each time. Donor platelets can vary greatly in their sensitivity to activation by HIT sera. One of the earliest and best-established activation assays, serotonin-release assay, involves ^{14}C-serotonin release from platelets induced by HIT antibodies and heparin. An activation assay will have greater specificity than the antigen assay, although specificity may vary based on the experience of the reference center. Its major usefulness is in post factum documentation of the cause of thrombocytopenia.
- Other activation tests include platelet activation in a heparin-induced platelet activation assay, luminography, and microparticle generation.
- A practical approach is that in patients with a high clinical risk of having HIT, heparin should be stopped and alternative treatment is started even before laboratory results are available. A positive antigen test and particularly a progressive increase in the number of platelets over the following days are confirmatory for HIT. A negative antigen test does not rule out HIT and should be repeated after 24 hours while the patient is undergoing alternative anticoagulant therapy. If the repeat assay is negative and platelet count does not increase, alternative diagnoses should be considered.
- Many patients with HIT often have complicated medical and surgical conditions, many of which can also cause thrombocytopenia. A differential diagnosis is listed in **Table 90–5**.

TABLE 90–5 ALTERNATIVE CAUSES OF CLINICAL CONDITIONS SIMULATING HEPARIN-INDUCED THROMBOCYTOPENIA

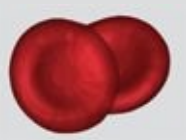
Thrombocytopenia
Increased destruction
• Acute immune thrombocytopenic purpura
• Dilutional thrombocytopenia
• Posttransfusion purpura
• Drug-induced thrombocytopenia

• Quinidine, quinine, trimethoprim-sulfamethoxazole, rifampicin, carbamazepine, diclofenac, ibuprofen • Integrin $\alpha_{IIb}\beta b_3$ inhibitors: abciximab, tirofiban, eptifibatide *Decreased production* • Chemotherapy • Malignancy • Drug-related • Thrombocytopenia and thrombosis • Consumptive thrombohemorrhagic disorders • Sepsis and disseminated intravascular coagulation • Malignancies • Disseminated intravascular coagulation in pregnancy or after snake bite • Thrombotic thrombocytopenic purpura • Hemolytic uremic syndrome • Systemic lupus erythematosus
Thrombosis alone • Venous stasis • Central catheters • Drugs • Coumadin • Vasculitis • Antiphospholipid syndrome

Treatment

- An established diagnosis or high clinical suspicion of HIT should lead to immediate termination of heparin treatment. Because patients remain at high risk for thrombotic complications, alternative anticoagulant treatment should be instituted.
- Coumadin should not be used as the sole initial treatment of HIT because of the increased risk of untoward thrombosis, in particular skin gangrene. Low-molecular-weight heparin should not replace high-molecular-weight heparin because of cross-reactivity. Synthetic pentasaccharides may be considered as alternative anticoagulant treatment, although there is a single publication describing cross-reactivity with HIT antibodies.
- Other accepted drugs that are used in the treatment of patients with HIT are danaparoid sodium (no longer available in the United States), recombinant hirudin (lepirudin), argatroban, desirudin, and bivalirudin.
- Lepirudin (limited availability) and argatroban directly inhibit thrombin. Both drugs are given intravenously and have rapid onset of action. Lepirudin binds to two sites on thrombin, the catalytic site and a fibrinogen-binding site, whereas argatroban binds only to the active site. Lepirudin prolongs the aPTT, so this test can be used to monitor effective dosing.
- Lepirudin is excreted in the urine, and its half life is dramatically increased in patients with renal failure. Lepirudin induces antilepirudin antibodies in approximately half of patients who receive the drug. These antibodies rarely alter biologic activity but do tend to prolong the drug's half-life, necessitating careful monitoring by aPTT.
- Argatroban is synthesized from arginine and is rapidly metabolized in the liver. It affects both the aPTT and PT.
- Use of lepirudin and argatroban in HIT is efficacious; the incidence of thrombotic complication is reduced, perhaps by half, and the time to platelet count recovery is shortened. However, bleeding complications can occur. Newer oral anticoagulant agents (eg, dabigatran, rivaroxaban, or apixaban) may prove to be effective, but clinical evidence is lacking thus far.

- As with danaparoid, lepirudin or argatroban should be given until patients recover from thrombocytopenia before adding and then switching to a prolonged course of an oral anticoagulant.



For a more detailed discussion, see Mortimer Poncz and Adam Cuker: Heparin-Induced Thrombocytopenia, Chap. 118 in *Williams Hematology*, 9th ed., and J. Evan Sadler Thrombotic Microangiopathies, Chap. 132 in *Williams Hematology*, 9th ed.

PART XII

TRANSFUSION AND HEMAPHERESIS

CHAPTER 91

Red Cell Transfusion

STORAGE AND PRESERVATION OF BLOOD

- Erythrocytes are preserved by liquid storage at 4°C or by frozen storage at either –80°C or –150°C.
- Preservative solutions for liquid storage all contain glucose, to provide energy, and citrate buffer at an acid pH to prevent coagulation by binding calcium and to counter the marked rise in pH that occurs when blood is cooled to 4°C.
- CPD-adenine is the preservative solution most frequently used in the United States at present. It contains adenine, citrate, phosphate, and dextrose (glucose).
- Adenine is added to help maintain intracellular levels of ATP.
- Erythrocytes are then separated and stored in an additive solution that contains glucose, adenine, and mannitol.
- The remainder of the blood collection is separated into plasma and platelets.
- Stored erythrocytes develop the so-called storage lesion, characterized in part by reduced levels of ATP, which interfere with glucose metabolism and reduce cell viability. 2,3-Bisphosphoglycerate levels also rapidly fall during storage, which increase the oxygen affinity of hemoglobin and thereby decreases the initial effectiveness of reinfused red cells. Potassium also leaks rapidly from stored cells.
- Frozen storage requires a cryoprotective agent to avoid hemolysis during freezing and thawing. Glycerol is the most frequently used agent. With proper technique, more than 80% of erythrocytes will survive frozen storage and function normally after transfusion.

WHOLE-BLOOD PREPARATIONS

- A unit of whole blood contains 435 to 500 mL of blood and 14 to 15 mL of preservative-anticoagulant solution for each 100 mL. Thus, if 450 mL of blood is collected, stored, and transfused, the patient will receive about 515 mL of total fluid.
- Blood collected in CDPA-1 (CDP with adenine) may be used after storage up to 35 days.
- There are very few, if any, indications for whole blood, and it is rarely used in modern transfusion practice.

FRESH BLOOD

- When blood is stored, platelet viability is lost within 48 hours, and the activity of coagulation factors V, VIII, and IX falls significantly.

- Thrombocytopenia and deficiency of the labile coagulation factors may occur in patients who receive transfusions of banked blood equal to their total blood volume in 24 hours.
- Fresh blood is often requested in an effort to avoid administration of blood deficient in these hemostatic components.
- It is better to treat such patients with a combination of packed red cells, fresh-frozen plasma, and platelet concentrates.
- Whole blood or packed red blood cells less than 5 to 7 days old should be transfused to patients with severe renal or hepatic disease or to newborns receiving exchange transfusion in order to avoid infusing excess free potassium.
- Patients who require massive transfusion should be given at least part of the transfusion as blood less than a few days old in order to avoid oxygen release problems caused by depletion of red cell 2,3-bisphosphoglycerate and to prevent replacement with platelet-poor blood.
- Patients with chronic transfusion-dependent anemia should probably receive blood less than 10 days old in order to maximize the interval between transfusions and to minimize iron accumulation.

PACKED RED BLOOD CELLS

- Packed red blood cells can be prepared from stored blood any time before the expiration date by centrifugation and removal of plasma to give a hematocrit of 60% to 90%.
- Red cells packed to a hematocrit of less than 80% can be stored until the expiration date of the original blood.
- Red cells, rather than whole blood, should be used for replacement of a red cell deficit.
- Packed red cells and electrolyte solutions are as effective as whole blood in replacing surgical blood loss.

LEUKOCYTE-POOR BLOOD

- Leukocyte-poor blood is best prepared by passing blood or packed cells through a special filter that removes leukocytes.
- It is used to prevent or avoid febrile reactions to leukocytes or platelets in previously sensitized patients; to minimize transmission of viral diseases, such as HIV or cytomegalovirus infections; and perhaps in patients awaiting kidney transplant.

WASHED RED CELLS

- Washed red cells are obtained from whole blood by centrifugation techniques.
- They must be used within 24 hours of preparation because of the danger of bacterial contamination.
- These cells are indicated for patients who are hypersensitive to plasma.
- They are sometimes used in neonatal transfusions to reduce the amount of anticoagulant, extracellular potassium, etc., infused.

FROZEN RED CELLS

- Frozen red cells may be stored for years but cost two to three times as much as stored liquid blood.
- They are somewhat leukocyte poor and almost free of plasma.
- They may be used for autotransfusion, to ensure a supply of rare blood, or to reduce sensitization to histocompatibility antigens in potential transplant patients.

INDICATIONS FOR TRANSFUSION THERAPY

- Informed consent should be obtained and documented before transfusion therapy is given.

Hemorrhage and Shock

- Volume support is of primary concern, but replacement of red cells is also necessary with larger losses of blood.
- Packed red cells with crystalloids or albumin are as effective as whole blood in replacing volume loss.

Surgery

- Blood loss (even >1000 mL) may be safely replaced with crystalloids.
- Because of the hazards of blood transfusion (see below), every effort should be made to minimize the use of blood for volume replacement in surgery.

Burns

- Severe burns require extensive volume replacement in the first 24 hours.
- Plasma loss occurs over the next 5 days and can be replaced with plasma and colloids.
- Anemia can be treated with packed red cells.

Anemia

- Patients with stable anemia with a hemoglobin level above 7 g/dL should not be transfused unless they are elderly or have cardiac or pulmonary disease.
- Attempts to improve the efficiency of transfusion by increasing the red blood cell circulation times by using young red cells (“neocytes”) have had limited success.

MODE OF ADMINISTRATION

- It is essential that the person administering blood or a blood component read the label to ensure that the unit to be used was selected by the laboratory for the particular patient.
- Usually blood does not need to be warmed when given to adults unless amounts greater than 3 L are to be given at greater than 100 mL/min. At the usual rate of administration, the aggregates that may develop in patients with high-titer cold agglutinins may be dispersed when the blood reaches body temperature.

- Blood being given to patients with cold agglutinins or cryoglobulinemia should be warmed to prevent further vascular damage.
- Blood should be given slowly in the first 30 minutes to minimize an adverse reaction.
- Drugs or medications should not be added to blood or blood components.

SPECIAL SITUATIONS

Autologous Transfusions

- Such transfusions minimize the probability of adverse reactions to transfusion, such as transmission of disease or alloimmunization.
- They may be achieved by preoperative collection and storage of blood, immediate preoperative phlebotomy and hemodilution with postoperative return of the blood, or reinfusion of blood collected intraoperatively.
- In some patients, erythropoietin has been given to permit increasing the amount of blood taken preoperatively. Approximately one additional unit of blood can be collected if the patient is supplemented with erythropoietin, making the actual benefit questionable.
- Autologous donation is ideal for patients with rare blood types or with antibodies that make cross-matching difficult or impossible.

Directed or Designated Donations

- Donors recruited from among family or friends are no safer than volunteer blood donors. Graft-versus-host disease in stem cell transplant recipients receiving transfused blood products is a greater risk if blood is donated by family members.

TRANSFUSION REACTIONS

- The majority of fatal transfusion reactions are due to management-clerical errors.
- Up to 20% of all transfusions may lead to some type of adverse reaction.

Immediate Reactions

Acute Hemolytic Reactions

- These reactions may occur intravascularly, usually because of ABO incompatibility, or extravascularly.
- Intravascular hemolysis may lead to disseminated intravascular coagulation (DIC) or to ischemic necrosis of tissues, particularly the kidney.
- Patients may develop fever, low back pain, sensation of chest compression, hypotension, nausea, or vomiting.
- The transfusion should be terminated immediately when an acute reaction is suspected, and measures to control hemorrhage, if present, and to prevent renal damage instituted promptly.
- Laboratory diagnosis is based on evidence of hemolysis (hemoglobinemia, methemalbuminemia, hemoglobinuria) and detection of a blood group incompatibility.
- Renal damage may be prevented by hydration with addition of a diuretic if necessary to

maintain urinary flow greater than 100 mL/h. Mannitol may be used at an initial dose of 100 mL of a 20% solution given intravenously over 5 minutes. Furosemide in a dose of 40 to 80 mg intravenously may be more effective.
- If oliguria occurs, standard measures for acute renal failure should be instituted.
- The risk of sequelae is dependent on the amount of incompatible blood given. Severe complications rarely occur if fewer than 200 mL of red cells have been transfused.

Febrile Reactions

- Fever may be caused by a hemolytic reaction, sensitivity to leukocytes or platelets, bacterial pyrogens, cytokines released by stored leukocytes or unidentified causes.
- Thirty percent of all transfusion reactions are nonhemolytic, febrile reactions.
- A febrile reaction of itself is not an indication for termination of the transfusion, but one should not hesitate to stop if there is any doubt about the cause.
- Chills may indicate a more serious situation, but there are no reliable guidelines.
- Sensitization to leukocyte or platelet antigens is a common cause of febrile reactions.
- At least seven transfusions are usually required for sensitization, but previously pregnant women may be sensitized after only one or two.
- Clinical findings are primarily fever, which may continue to rise for 2 to 6 hours after the transfusion is stopped and may continue for 12 hours.
- Diagnosis depends on demonstration of antibodies to leukocyte or platelet antigens. Most reactions are a result of antibodies to granulocytes.
- Treatment is supportive.
- Many reactions can be prevented by use of a leukocyte filter, especially if applied to the unit of blood shortly after collection.

Transfusion-Related Acute Lung Injury

- Transfusion-related acute lung injury (TRALI) is a syndrome of acute hypoxia as a result of noncardiogenic pulmonary edema that follows transfusion. All blood components have been implicated in TRALI, but most frequent are plasma-containing products, which account for 50% to 63% of TRALI fatalities.
- The precise mechanisms of the capillary leak syndrome in TRALI have not been fully determined, but two main hypotheses have been proposed. One involves white cell antibody-mediated TRALI and the other cytokine-mediated TRALI.
- It is often impossible to distinguish TRALI from acute respiratory distress syndrome. The typical presentation of TRALI is the sudden development of dyspnea, severe hypoxemia (O_2 saturation <90% in room air), hypotension, and fever that develop within 6 hours after transfusion and usually resolve with supportive care within 48 to 96 hours. Although hypotension is considered one of the important signs in diagnosing TRALI, hypertension can occur in some cases.

Pulmonary Hypersensitivity Reaction (Noncardiogenic Pulmonary Edema)

- Leukocyte incompatibility may also cause acute respiratory distress, chills, fever, and tachycardia as a consequence of pulmonary edema.

- Donor leukocytes may react with recipient antibodies, or donor antibodies may react with recipient leukocytes.
- Almost 25% of multiparous women have antibodies that can cause this reaction.
- This reaction can occur with transfusion of platelets, plasma, whole blood, or packed red cells.
- Onset is usually within 4 hours of transfusion.
- Chest films show bilateral diffuse, patchy pulmonary densities without cardiac enlargement.
- Treatment is supportive.
- In a healthy individual, symptoms subside in less than 24 hours, and the pulmonary infiltrates disappear within 4 days.

Allergic Reactions

- Transfusion may result in generalized pruritus and urticaria, and occasionally there may be bronchospasm, angioedema, or anaphylaxis.
- The cause is poorly understood, but may be hypersensitivity to plasma proteins or other substances in the administered product.
- These reactions are usually mild and respond to antihistamine drugs, but epinephrine may be required in some cases.

Anti-IgA in IgA-Deficient Recipient

- Severe anaphylactic reactions may occur in IgA-deficient patients who have formed anti-IgA antibodies.
- Deficiency or absence of IgA occurs in about 1 in 800 people.
- IgA in the transfused product reacts with circulating antibody in the recipient. Less than 10 mL plasma can cause a reaction.
- Symptoms are dyspnea, nausea, chills, abdominal cramps, emesis, diarrhea, and profound hypotension. There is no fever.
- Diagnosis depends on demonstration of IgA deficiency and anti-IgA antibodies in the recipient.
- Reactions can usually be prevented by using washed red cells. Platelet or granulocyte transfusions for sensitized patients should be from donors with absent IgA.

Bacterial Contamination

- Blood may be contaminated by cold-growing organisms (*Pseudomonas* or coli-aerogenes group) that utilize citrate and may therefore lead to formation of visible clots.
- Infusion of blood containing large numbers of gram-negative organisms leads to endotoxin shock, with fever, hypotension, abdominal pain, vomiting, diarrhea, and vascular collapse, beginning immediately after infusion is started or 30 minutes or more after the infusion.
- Diagnosis may be made by examining a Gram stain of plasma obtained by low-speed centrifugation of some of the transfused blood. If the blood is heavily contaminated, organisms should be seen in every oil immersion field.
- Bacterial contamination of blood is uncommon if disposable plastic blood bags are used, but contamination may be a significant hazard with platelet concentrates stored at room temperature.

Circulatory Overload

- Congestive heart failure with pulmonary edema may develop following transfusion in patients with cardiovascular compromise. Treatment is primarily with diuretics.
- Patients with severe chronic anemia may also develop congestive heart failure if transfused rapidly. Diuretics should be given and the transfusion limited to 2 mL/kg per hour.

Microaggregates in Blood

- Particles of 13 to 100 microns in size ("microaggregates") and consisting largely of platelets and fibrin in banked blood are not removed by the usual filters in transfusion sets.
- Such particles can cause pulmonary insufficiency when massive transfusion of banked blood is given using standard filters, but this can be prevented with microaggregate filters.

Citrate Intoxication

- Blood transfused into adults at a rate greater than 1 liter in 10 minutes will cause significant reduction in ionized calcium concentrations and lead to myocardial depression and electrocardiographic changes.
- This can be prevented by giving 10 mL of 10% calcium gluconate intravenously for every liter of citrated blood administered.

Delayed Reactions

Delayed Hemolytic Reaction

- Previously undetected alloantibodies may appear 4 to 14 days after a first transfusion and cause destruction of the transfused cells. An anamnestic response immediately following transfusion can occur in patients who had been previously transfused or pregnant if the relevant antigen is presented to the patient.
- Clinical findings are jaundice, falling hemoglobin level, and a positive direct antiglobulin reaction (Coombs test). The blood bank can usually differentiate if a positive Coombs test is from alloantibodies or from non–transfusion-related autoantibodies.
- Delayed hemolytic reactions may be mild and probably are frequently undetected.

Post-transfusion Purpura

- Thrombocytopenia caused by antibodies to a platelet-specific antigen may develop shortly after transfusion (see Chap. 73).

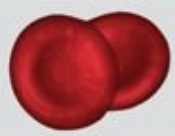
Transmission of Disease

- The greatest risks are viral agents such as hepatitis B or C or HIV, although with current blood donor screening procedures the risk of each of these infections is less than 1:1,000,000.

Other Adverse Effects

- Graft-versus-host disease is an uncommon complication of transfusion, preventable by administering irradiated blood.

- Iron overload may occur in patients who require chronic transfusion therapy (see Chap. 9).
- Alloimmunization to antigens not included in routine cross-matching occurs in immunocompetent patients receiving multiple transfusions and creates a major problem in obtaining blood for some patients with chronic anemia.



For a more detailed discussion, see Jeffrey McCullough, Majed A. Refaai, and Claudia S. Cohn: Blood Procurement and Red Cell Transfusion, Chap. 138 in *Williams Hematology*, 9th ed.

CHAPTER 92

Transfusion of Platelets

PLATELET PRODUCTS FOR TRANSFUSION

- Random donor platelets are prepared by centrifugation techniques that yield from 7 to 10 × 10^{10} platelets per unit of blood.
- Platelets so obtained are suspended in citrated autologous plasma and are significantly contaminated with leukocytes. Several units of platelets are pooled to provide sufficient platelets for transfusion (4–6 U for an adult).
- Single-donor platelets are prepared from a single individual by plateletpheresis. Each plateletpheresis contains approximately 3 to 4 × 10^{11} platelets, significantly contaminated with leukocytes.
- Fresh whole blood is used for platelet transfusion in children younger than 2 years of age who have undergone open heart surgery.

STORAGE OF PLATELET CONCENTRATES

- Platelet suspensions may be stored with continuous agitation for 5 days at 20°C to 24°C in plastic containers, which allow for adequate diffusion of oxygen.
- In vivo function of stored platelets is nearly normal.
- Platelets may be stored frozen in plasma containing dimethyl sulfoxide (DMSO).
- Viability of thawed platelets is 50% that of fresh platelets.
- Frozen storage is usually used to provide autologous platelets for use in patients who are refractory to allogeneic platelet transfusions.

CHOICE OF A PLATELET PREPARATION

- Platelet transfusion may begin with random-donor pooled platelets. However, single-donor platelets are a better product with less risk of transmission of infectious agents. As such, whole blood–derived platelet use has fallen to 15% to 20% of the platelet doses transfused in the United States because of blood center convenience (no need to separate from whole blood) and the superiority of single donor platelets.
- ABO-compatible platelets should be used whenever possible.

CLINICAL RESPONSE AND COMPLICATIONS OF PLATELET TRANSFUSION

- The response to infusion of random donor platelets can be evaluated by calculating the *corrected count increment (P)*:

$$p = C \times S/U \text{ (platelets/L)}$$

  Where C = measured platelet increase (platelets/L)
  S = body surface area in square meters
  U = number of units of platelet given
- Average corrected count increment is 10×10^9/L.
- In a single-donor plateletpheresis product, there are about the same number of platelets as in five random-donor units.
- The 20-hour increment is two-thirds of the 1-hour increment under normal conditions (absence of alloimmunization, ongoing hyperconsumption of disseminated intravascular coagulation or bleeding, or pooling in an enlarged spleen).
- Additional factors that lower the corrected count increment are loss of platelet viability in storage, stem cell transplantation, or drug therapies (eg, amphotericin).

Alloimmunization

- Alloimmunization frequently develops in patients receiving random-donor platelet transfusions.
- It should be considered if two to three consecutive random donor transfusions produce a corrected count increment of less than 3×10^9/L.
- It is usually caused by development of antibody against human leukocyte antigen (HLA) antigen on the platelet surface. Leukocyte depletion of platelet products may reduce alloimmunization.
- Patients may respond to single-donor platelets from either family members or unrelated individuals selected by matching for the HLA-A and B antigens.
- Rh-negative recipients may become sensitized to Rh-positive red cells contaminating infused platelets.
- During and after platelet transfusion, chills and fever may occur from alloantibodies against contaminating leukocytes.
- Leukocyte depletion reduces the frequency of chills and fever.
- Febrile reactions may be caused by allergic reactions to some component(s) of the suspending plasma.
- Graft-versus-host disease may occur in immunosuppressed patients given unirradiated platelet transfusions.

Transmission of Microorganisms

- Chills immediately on infusion of platelets suggest bacterial contamination of the unit.
- Contamination of stored platelets by bacteria is much more common than contamination of other blood products; because they are stored at room temperature and because of their normally turbid appearance, an infected platelet unit may not appear physically different from a normal unit.
- Platelet transfusion can transmit viruses (eg, hepatitis B and C, HIV, and cytomegalovirus).

INDICATIONS FOR PLATELET TRANSFUSION

- Platelet counts of greater than 5 to 10 × 10^9/L are adequate to protect patients against life-threatening spontaneous bleeding.
- Invasive procedures may require raising the platelet count to approximately 60 × 10^9/L.
- ∈-Aminocaproic acid (3–5 g orally every 6 hours) can reduce mucosal bleeding in thrombocytopenic patients.

Thrombocytopenia as a Result of Underproduction

- Platelets should be transfused prophylactically for a platelet count of 5 × 10^9/L or less, unless the patient has little hope of significant recovery from the underlying cause of thrombocytopenia, in which case bleeding should inform the decision to transfuse.
- Transfusion to maintain platelet counts greater than 20 × 10^9/L without regard to special circumstances has no support from clinical studies and results in waste of platelets and unnecessary risks to patients.
- The decision whether to transfuse platelets in the range of 5 to 20 × 10^9/L must be made on an individual basis using clinical considerations such as the presence of fever and sepsis, the presence of gastrointestinal ulceration or bleeding, the administration of drugs that interfere with platelet function, abnormalities of coagulation factors, and/or a very high leukocyte count.

Thrombocytopenia Caused by Platelet Loss, Sequestration, or Destruction

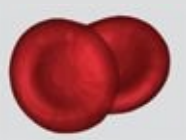
- Massive red blood cell transfusion only rarely requires prophylactic platelet transfusion unless there is abnormal bleeding.
- Prophylactic platelet transfusion is not indicated for the thrombocytopenia that develops after cardiopulmonary bypass unless there is abnormal bleeding.
- Thrombocytopenia from splenomegaly and sequestration of platelets does not usually require prophylactic platelet transfusion unless an invasive procedure is to be done.
- Patients with immune thrombocytopenia do not usually require platelet transfusion.
- If bleeding is life-threatening, 3 to 6 units of random-donor platelets per square meter of body surface area may raise the platelet count for 12 to 48 hours.
- The same considerations apply for other disorders with accelerated destruction of platelets, (eg, thrombotic thrombocytopenic purpura, disseminated intravascular coagulation).
- Transfusion of washed maternal platelets to an infant is indicated in neonatal alloimmune thrombocytopenia. Unfortunately, arranging for apheresis of the mother often is difficult, so transfusion of platelet concentrates to neonates who are severely thrombocytopenic or bleeding is appropriate and lifesaving. It is not appropriate to wait for the laboratory confirmation of the diagnosis in suspected cases.

Qualitative Platelet Disorders

- Platelet transfusion is not indicated for extrinsic platelet disorders (eg, uremia, von Willebrand disease, hyperglobulinemia).
- Inherited intrinsic platelet disorders are often mild and do not require platelet transfusion

except for severe bleeding and surgery.

- Acquired intrinsic platelet disorders usually do not require platelet transfusion unless the patient is also thrombocytopenic.



For a more detailed discussion, see Terry Gernsheimer and Sherril Slichter: Preservation and Clinical Use of Platelets, Chap. 139 in *Williams Hematology*, 9th ed.

CHAPTER 93
Therapeutic Hemapheresis

- Therapeutic apheresis is the application of blood cell separation techniques to treat certain clinical conditions.
- A continuous-flow blood separator is usually used.
- **Table 93–1** contains the principal applications of the technique.
- Hemapheresis is usually used in hematologic therapy for acute problems.
- Adverse effects are infrequent and mild; they are hypotension, urticaria, and hypocalcemia.
- Cytapheresis refers to removal or exchange of a blood cell element (eg, leukapheresis, plateletpheresis, erythrocytapheresis).
- Plasmapheresis refers to removal or exchange of plasma.

TABLE 93–1 THERAPEUTIC HEMAPHERESIS TECHNIQUES

Cell depletion
Plateletpheresis
Leukapheresis
Blood component exchange
Plasma exchange (plasmapheresis)
Red cell exchange
Blood component modification
Selective extraction of a plasma constituent
Photopheresis

PLATELETPHERESIS

- Thrombocythemia or extreme thrombocytosis can usually be managed pharmacologically.
- Plateletpheresis is useful for those who need rapid, temporary reduction of the platelet count in emergent conditions (eg, ongoing thrombosis) or for patients who cannot tolerate drug therapy (eg, early pregnancy).
- If plateletpheresis is required in patients with thrombocythemia requiring urgent platelet reduction, pharmacologic therapy should be administered simultaneously for long-term control (see Chap. 42).
- Reduction in the platelet count of about 50% may be achieved with each procedure, but the platelet count returns to pretreatment value in a few days.

LEUKAPHERESIS

- Leukostasis may be ameliorated by leukapheresis with rapid cytoreduction in patients with acute myelogenous leukemia whose leukocyte count is greater than 50 to 100 × 10^9/L; patients

with acute lymphocytic leukemia whose leukocyte count is greater than 75 to 100 × 10^9/L; or patients with chronic myelogenous leukemia (CML) whose leukocyte count is greater than 300 × 10^9/L, or who have > 50 × 10^9/L blasts.

- Unfortunately, there are no clearly established thresholds, so that patients with any blast count who have signs of leukostasis should undergo leukapheresis.
- Therapeutic leukapheresis prior to chemotherapy reduces tumor burden and may minimize metabolic abnormalities due to tumor lysis (see Chap. 46).
- Therapeutic leukapheresis can lower the white cell counts, reduce organomegaly, and reduce tumor burden in chronic lymphocytic leukemia, but cytotoxic therapy is required for disease control.
- Therapeutic leukapheresis may be used in lieu of chemotherapy to treat CML (eg, in pregnancy), to allow for delay in starting chemotherapy until after the first trimester or longer.
- In acute or chronic leukemia, a single therapeutic leukapheresis will reduce the leukocyte count by 25% to 50%.
- The rate of mobilization of cells and the rate of cell proliferation dictate the frequency of therapeutic leukapheresis required to achieve goal.
- Photopheresis, extracorporeal photochemotherapy, can improve erythroderma in cutaneous T-cell lymphoma (Sézary syndrome). Leukocytes removed by cytapheresis are treated with 8-methoxypsoralen and ultraviolet light and returned to patient (see Chap. 65).
- Leukapheresis can be used to harvest lymphocytes, dendritic cells, or allogeneic or autologous blood stem cells for immunotherapy or stem cell transplantation.

ERYTHROCYTAPHERESIS (RED CELL EXCHANGE)

- Red cell exchange carries the same potential hazards as blood transfusion.
- Indications for red cell exchange in sickle cell disease include priapism, unremitting painful crises, acute chest syndrome, stroke, and prior to radiographic studies requiring hyperosmolar contrast medium. Its use during pregnancy, for chronic painful crises, and prior to surgery is controversial (see Chap. 16).
- Acute neurologic symptoms have occurred in sickle cell anemia patients undergoing red cell exchange for priapism.
- Red cell exchange has been used to decrease parasite load in severe falciparum malaria and extreme polycythemia.

PLASMA EXCHANGE THERAPY

- Plasma exchange is used in disorders in which there is a known or presumed abnormal plasma constituent to remove pathologic material in the plasma (eg, thrombotic thrombocytopenic purpura [see Chap. 90] or hyperviscosity syndrome in Waldenström macroglobulinemia [see Chap. 69]).
- An exchange of one plasma volume reduces the abnormal plasma constituent by approximately 65%, and an exchange of two plasma volumes reduces the abnormal plasma constituent by approximately 88%.

- Alterations in plasma components after plasma exchange include reduced levels of coagulation factors after large volume exchange and replacement with albumin and crystalloid, but bleeding is rare. Factor levels are restored over next 72 hours; serum immunoglobulin levels are decreased after repeated one-volume plasma exchanges and replacement with albumin. It takes several weeks for levels to return to normal.
- Mortality associated with the procedure of plasma exchange is less than 3 in 10,000 procedures with today's technology.
- **Table 93–2** lists disorders for which plasma exchange may be useful. These conditions include thrombotic thrombocytopenic purpura, renal failure associated with multiple myeloma, and hyperviscosity syndrome due to paraproteins (especially macroglobulinemia, cold agglutinin disease with severe hemolysis not responding to other measures, cryoglobulinemia with vasculitis, glomerulonephritis, severe Raynaud syndrome, removal of coagulation factor inhibitors, recipients of ABO-incompatible marrow transplants prior to transplantation, posttransfusion purpura).
- See **Table 93–3** for other antibody-mediated disease indications.

TABLE 93–2 INDICATION CATEGORIES FOR THERAPEUTIC APHERESIS ACCORDING TO THE AMERICAN SOCIETY FOR APHERESIS

Category	Definition of Category
I	Apheresis is an accepted first-line therapy for these disorders.
II	Apheresis is an accepted second-line therapy for these disorders.
III	Individualize decision making. The optimal role of apheresis has not been conclusively determined in these disorders.
IV	Published evidence indicates that apheresis is ineffective or harmful in these disorders. Seek institutional review board approval if apheresis is planned.

Adapted with permission from Schwartz J, Winters JL, Padmanabhan A, et al. Guidelines on the use of therapeutic apheresis in clinical practice-evidence-based approach from the Writing Commit-tee of the American Society for Apheresis: the sixth special issue, *J Clin Apher* 2013 Jul;28(3):145–284.

TABLE 93–3 INDICATIONS FOR APHERESIS IN HEMATOLOGY: INDICATION CATEGORY ASSIGNMENTS AND RECOMMENDATION

Clinical Disorder	Apheresis Procedure*	Indication Category†	Grade‡ of Recommendation
Amyloidosis, systemic	TPE	IV	2C
Aplastic anemia or pure red cell aplasia	TPE	III	2C
Autoimmune hemolytic anemia (warm)	TPE	III	2C
Babesiosis, severe Babesiosis, high-risk population	RBC exchange	I II	1C 2C
Catastrophic antiphospholipid syndrome	TPE	II	2C
Coagulation factor inhibitor			
Alloantibody	TPE	IV	2C
Alloantibody	IA	III	2B
Alloantibody	TPE	III	2C
Alloantibody	IA	III	1C

Cold agglutinin disease	TPE	II	2C
Cryoglobulinemia	TPE IA	I II	2A 2B
Cutaneous T-cell lymphoma; mycosis fungoides; Sézary syndrome (erythrodermic)	ECP	I	1B
Erythrocytosis Primary (polycythemia vera) Secondary	Erythrocytapheresis	I III	1B 1C
Graft-versus-host disease, skin Chronic Acute	ECP	II II III	1B 1C 2C
Graft-versus-host disease, nonskin (acute/chronic)			
Hemopoietic stem cell transplant, ABO incompatible			
Major incompatibility, marrow	TPE	II	1B
Major incompatibility, apheresis	TPE	II	2B
Minor incompatibility, apheresis	RBC exchange	III	2C
Hemolytic uremic syndrome Atypical Complement gene mutations Factor H antibodies MCP mutations	TPE	II I IV IV III	2C 2C 1C 1C 2C
Infection-associated Shiga toxin–associated Streptococcal pneumonia–associated			
Heparin-induced thrombocytopenia			
Precardiopulmonary bypass	TPE	III	2C
Thrombosis		III	2C
Hereditary hemochromatosis	Erythrocytapheresis	I	1B
Hyperleukocytosis (acute leukemia)			
Leukostasis	Leukocytapheresis	I	1B
Prophylaxis		III	2C
Hyperviscosity in monoclonal gammopathies			
Symptomatic	TPE	I	1B
Prophylaxis for rituximab		I	1C
Immune thrombocytopenia (refractory)	TPE IA	IV III	2C 2C
Myeloma cast nephropathy	TPE	II	2B
Posttransfusion purpura	TPE	III	2C
Sickle cell disease			
Acute stroke	RBC exchange	I	1C
Acute chest syndrome		II	1C
Multiorgan failure		III	2C
Preoperative management		III	2A
Priapism		III	2C

Sequestration syndrome (spleen, liver, cholestasis)		III	2C
Stroke prophylaxis		II	1C
Vasoocclusive pain		III	2C
Thrombocytosis			
Symptomatic	Thrombocytapheresis	II	III
Prophylaxis (or secondary)		2C	2C
Thrombotic microangiopathy			
Hemopoietic stem cell transplant-related	TPE	III	2C
Drug associated		I	1B
Ticlopidine		III	2B
Clopidogrel		III	2C
Calcineurin inhibitors		IV	2C
Gemcitabine		IV	2C
Quinine		I	1A
Thrombotic thrombocytopenic purpura			

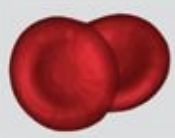
*Apheresis procedures: ECP, extracorporeal photochemotherapy (photopheresis); IA, immunoadsorption apheresis; MCP, monocyte chemoattractant protein; TPE, therapeutic plasma exchange.

Note: Leukocytapheresis, erythrocytapheresis, and thrombocytapheresis refer to removal of white cells, red cells, or platelets, respectively, by apheresis. Red blood cell exchange refers to apheresis removal of red blood cells and simultaneous replacement of removed red cells with donor red blood cells.

†Indication categories: see Table 93-2 for indication category.

‡Grade of recommendation: 1 = strong recommendation (ie, “we recommend”); 2 = weak recommendation (ie, “we suggest”). A = recommendation based on high-quality published evidence; B = based on moderate-quality published evidence; C = based on low-quality published evidence.

Adapted with permission from Schwartz J, Winters JL, Padmanabhan A, et al. Guidelines on the use of therapeutic apheresis in clinical practice-evidence-based approach from the Writing Commit-tee of the American Society for Apheresis: the sixth special issue, *J Clin Apher* 2013 Jul;28(3):145-284.



For a more detailed discussion, see Robert Weinstein: Principles of Therapeutic Apheresis: Indications, Efficacy, and Complications, Chap. 28 in *Williams Hematology*, 9th ed.

Table of Normal Values

Laboratory Variables Relevant to Hematologic Diagnosis (Normal Adult Values)

BLOOD CELLS		
Variable (Common Abbreviations)	**Units**	**Values**
Hematocrit (HCT) or Packed cell volume (PCV)	Percent or mL red cells/dL blood, respectively	M = 42–51 F = 36–46
Hemoglobin (Hb, Hgb)	g/dL blood	M = 14–18 F = 12–15
Red cell count (RBC, RCC)	10^6/μL or 10^{12}/L	M = 4.5–6.0 F = 4.1–5.1
Mean cell volume (MCV)	fL/cell	M = 80–96 F = 79–94
Mean cell hemoglobin (MCH)	pg/cell	27–33
Mean cell hemoglobin concentration (MCHC)	g/dL red cells	33–36
Red cell distribution width (RDW)	Percent	< 15
Reticulocyte count	Percent of red cells	0.5–1.5
Reticulocyte hemoglobin (CHr)	pg/cell	27–33
Total blood volume (TBV)	mL/kg	65–85[+]; 55–75[#]
Plasma volume (PV)	mL/kg	39–44
Red cell mass (RCM)	mL/kg	25–35
Platelet count	10^3/μL or 10^9/L	175–450
White cell count (WBC, WCC)	10^3/μL or 10^9/L	4.8–10.8
Absolute monocyte count	10^3/μL or 10^9/L	0.3–0.8
Absolute neutrophil count	10^3/μL or 10^9/L	1.8–7.7
Absolute lymphocyte count	10^3/μL or 10^9/L	1.0–4.8
CD3-positive lymphocytes	10^3/μL or 10^9/L	700–1900
CD4-positive lymphocytes	10^3/μL or 10^9/L	400–1400
CD8-positive lymphocytes	10^3/μL or 10^9/L	200–700
CD19-positive lymphocytes	10^3/μL or 10^9/L	50–375
HEMOGLOBIN ELECTROPHORESIS*		
Hemoglobin A1	Percent of total hemoglobin	96.1–99.0

Hemoglobin A2	Percent of total hemoglobin	0.8–3.4
Hemoglobin F	Percent of total hemoglobin	0.0–1.2
COAGULATION TESTS		
Prothrombin time (PT)	Seconds to clot	12–14
International Normalized Ratio (INR)	None	0.8-1.2
Partial thromboplastin time (PTT)	Seconds to clot	19–30
Thrombin time	Seconds to clot	10–15
Closure time (PFA-100) Collagen/epinephrine (CEPI)	Seconds	<175
Clot retraction	Percent in 1 hour	>40
Fibrinogen	mg/dL plasma	188–381
D-Dimer	ng/mL	<400
Factor II, V, and VII	Percent of normal mean	50–150
Factor VIII: c activity	Percent of normal mean	50–200
Willebrand factor activity	Percent of normal mean	60–200[§]
Willebrand factor antigen	Percent of normal mean mg/L	50–160[§] ~ 100
Factor VIII-inhibitor	Bethesda units	0–0.5
Factor IX	Percent of normal mean Mg/L	50–150 ~ 4.0
Factor X	Percent of normal mean mg/L	50–150 ~ 10
Factor XI	Percent of normal mean mg/L	50–150 ~ 7.0
Factor XII	Percent of normal mean	50–150
Factor XIII	Percent of normal mean	70–130
α2- antiplasmin	Percent of normal mean	80–120
Plasminogen	Percent of normal mean	80–120
Antithrombin: Functional assay Immunologic assay	Percent of normal mean mg/dL	80–120 22–33
Protein C	Percent of normal mean µg/mL	70–140 3.0–5.0
Activated protein C resistance	APC ratio	>1.5
Protein S Total Free Free/total ratio	Percent of normal mean µg/mL µg/mL Unitless	65–140 20–25 6–10 ~ 0.4
Fibrin degradation products (latex particles)	µg/mL	<20
Platelet Aggregation (in platelet-rich plasma) With collagen (2 µg/mL) With arachidonic acid (0.5 mM) With ADP 5 µM With ADP 10 µM With epinephrine (5 µM) With ristocetin (1.0 mg/mL)	Percent of control Percent of control Percent of control Percent of control Percent of control Percent of control	70–95 70–100 70–90 70–90 75–90 60–80

Platelet ATP release (in blood)	nmoles of ATP	>0.5
With thrombin (1 unit)	nmoles of ATP	0.5–1.7
With collagen (2 mg/mL)	nmoles of ATP	0.9–1.7
(5 mg/mL)	nmoles of ATP	0.56–1.4
With arachidonic acid (0.5 mM)	nmoles of ATP	0–0.7
ADP (5 mM)	nmoles of ATP	0.38–1.71
ADP (19 mM)		
RELEVANT BLOOD CHEMISTRIES		
Serum haptoglobin	mg/dL	30–200
Serum iron	µg/dL	M = 75–175
	µmol/L	M = 13–31
	µg/dL	F = 65–165
	µmol/L	F = 11–29
Serum total iron	µg/dL	260–420
binding capacity	µmol/L	44–80
Saturation of total iron binding capacity	Percent	15–45
Serum ferritin	ng/mL or µg/L	M = 15–250
		F = 11–125
		F > 40 yrs = 12–250
Serum soluble truncated transferrin	mg/L	1.0–3.7
receptor (sTfR)	nmol/L	9–28
Serum folate	nmol/L	7–45
Red cell folate	nmol/L	300–1000
	ng/mL	130–475
Serum Vitamin B_{12}	pg/mL	200–1000
Serum Erythropoietin (adults)	mU/mL	4–19

Abbreviations: kg = kilograms, g = grams, mg = milligrams, µg = micrograms, pg = picograms, ng = nanograms, L = liter, dL = deciliter, mL = milliliters, µL = microliter, fL = femtaliter, nmol = nanomoles, U = units, ADP = adenosine diphosphate, ATP = adenosine triphosphate.

*Adult levels reached by about 1 year. Newborns have 60–80% HbF and 20 to 40% of Hb A1.

+TBV derived from measurement of plasma volume.

TBV derived from measurement of red cell mass.

§May be as much as 20% lower in Blood type O individuals.

Values in infancy and childhood are not included (see Chap. 1 of this Manual and Chap. 7 of *Williams Hematology,* 9th Edition, 2016). The normal ranges described here are guidelines. They may vary from laboratory to laboratory and some lower and upper limits are still disputed. They vary based on reagents, assay and instruments used. Normal values should be established in the laboratory of record. This requirement is especially true of determinations such as prothrombin and thromboplastin time, D-dimer assay, platelet aggregometry, platelet ATP release, and others. Normal hemoglobin and hematocrit may be higher for persons living at altitude. Reticulocyte count may be reported as absolute number per liter. The conversion to the reticulocyte index as a measure of the marrow response to anemia is described in Chap. 1 of this Manual.

See also: Jacobs DS, Oxley DK, DeMott WR,: *Laboratory Test Handbook: Concise with Disease Index,* Lexi-Comp, Inc, 2004; Sacher RA, McPherson RA: *Widmann's Clinical*

Interpretation of Laboratory Tests, 11th Edition, F.A. Davis, Co., 2000; Burtis CA, Ashwood ER, Bruns DE: *Tietz Textbook of Clinical Chemistry:* 4th Edition, W.B. Saunders Co., 2005.

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